# A CORRESPONDING STATES TREATMENT OF THE SPEED OF SOUND IN SIMPLE LIQUIDS

By S. D. Hamann*

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*Summary*

It is shown that the speed of sound in the simple liquids A, $N_2$, $O_2$, $CH_4$ conforms to the principle of corresponding states as formulated by de Boer in molecular units. The lighter liquids $H_2$ and He show negative deviations which are proportional to their quantal parameters $\Lambda^*$. $CCl_4$ shows a positive deviation which is most likely due to a difference in the form of its intermolecular potential from that of the other liquids.

A simple theory is presented for the speed of sound in a hypothetical liquid at absolute zero. The calculated value is consistent with the experimental data at finite temperatures.

## I. Introduction

At low frequencies and low amplitudes, the speed of sound $u$ in a fluid is a simple thermodynamic property of the material. It is related to the coefficient of adiabatic compressibility $\varkappa_S$ and to the density $\rho$ by

$$u=1/(\varkappa_S\rho)^{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Alternatively, it can be expressed in terms of the isothermal compressibility $\varkappa_T$ and $\rho$ by

$$u=\gamma^{\frac{1}{2}}/(\varkappa_T\rho)^{\frac{1}{2}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where $\gamma$ denotes the ratio of the two specific heats of the fluid ($\gamma=C_P/C_V$). If the fluid is a pure substance of molecular weight $M$, of molar volume $V$, and is at a pressure $P$, then :

$$\rho=M/V, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

$$\varkappa_S=-\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_S, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

$$\varkappa_T=-\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

so that

$$u=V\left[-\frac{1}{M}\left(\frac{\partial P}{\partial V}\right)_S\right]^{\frac{1}{2}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

$$=V\left[-\frac{\gamma}{M}\left(\frac{\partial P}{\partial V}\right)_T\right]^{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

* C.S.I.R.O. Chemical Research Laboratories, Division of Physical Chemistry, High Pressure Laboratory, University of Sydney.

For an ideal gas, equation (7) reduces to

$$u=\left(\frac{\gamma \mathbf{R}T}{M}\right)^{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

This last relation requires that the speed of sound should be proportional to the square root of the absolute temperature. It holds in non-dissociating gases at low pressures. But it fails badly in highly compressed gases and in liquids, where the speed of sound often *decreases* with increasing temperature. The reason for this failure is that the compressibility of a dense material is very far from being ideal. It is governed, not by Boyle's law, but by the forces acting between the molecules of the material.

It is the purpose of the present paper to show that the speed of sound in simple liquids can be related to the intermolecular forces in a general way by invoking the theorem of corresponding states.

## II. Intermolecular Forces and Corresponding States in Simple Liquids

The liquefied inert gases are amongst the simplest liquids because their molecules are monatomic, non-polar, and spherically symmetrical. The interaction energy $\varepsilon$ between an isolated pair of such molecules depends only upon the distance $r$ between their centres, and it is well represented by the empirical formula :

$$\varepsilon=a/r^n-b/r^m, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

$$=\varepsilon_0\left[\frac{m}{n-m}\left(\frac{r_0}{r}\right)^n-\frac{n}{n-m}\left(\frac{r_0}{r}\right)^m\right], \quad \ldots\ldots\ldots \quad (10)$$

$$n>m,$$

where $-\varepsilon_0$ is the minimum mutual potential energy (relative to an energy zero at infinite separation) corresponding to the separation $r=r_0$. It seems that the exponents $n$ and $m$ are close to 12 and 6, respectively, for the inert gas molecules (Corner 1948) so that equation (10) becomes

$$\varepsilon=\varepsilon_0\left[\left(\frac{r_0}{r}\right)^{12}-2\left(\frac{r_0}{r}\right)^6\right]. \quad \ldots\ldots\ldots\ldots \quad (11)$$

It has been found that this potential applies also to slightly more complex molecules such as $H_2$, $N_2$, $CH_4$. The molecular parameters $\varepsilon_0$ and $r_0$ are characteristic of the particular gas and can be derived from the temperature dependence of its second virial coefficient (see for example, Fowler and Guggenheim 1949 ; Hirschfelder, Curtiss, and Bird 1954, p. 166).

de Boer and Michels (1938) and de Boer (1940, 1948) have shown that the principle of corresponding states can be formulated in terms of the parameters

$\varepsilon_0$ and $r_0$ by expressing the pressure, volume, and temperature in the molecular units:

Pressure

$$P_0 = 2^{\frac{1}{2}}\varepsilon_0/r_0^3, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (12)$$

Volume

$$V_0 = \mathbf{N}r_0^3/2^{\frac{1}{2}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (13)$$

Temperature

$$T_0 = \varepsilon_0/\mathbf{k}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (14)$$

where **N** is Avogadro's number and **k** is Boltzmann's constant. The quantities reduced in this way are conveniently denoted by asterisks, thus: $P^* = P/P_0$, $V^* = V/V_0$, $T^* = T/T_0$. de Boer also established by quantum statistics that a group of substances whose internal interaction energies are the sums of pair terms of the type (**11**) will obey a general equation of state:

$$P^* = f_1(V^*, T^*, \Lambda^*), \quad \ldots\ldots\ldots\ldots\ldots \quad (15)$$

where $f_1$ is a universal function and $\Lambda^*$ is a quantal parameter defined by

$$\Lambda^* = 2^{\frac{1}{2}}\mathbf{h}/r_0(m\varepsilon_0)^{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots\ldots \quad (16)$$

$\Lambda^*$ represents the reduced value of the de Broglie wavelength of relative motion of two molecules of mass $m$ ($=M/\mathbf{N}$) and relative kinetic energy $\varepsilon_0$, **h** being Planck's constant. It is a characteristic parameter for each substance and the larger its value the more will the substance deviate from classical behaviour. Some values of $\Lambda^*$, $\varepsilon_0$, $r_0$, $P_0$, $V_0$, $T_0$ are presented in Table 1.

TABLE 1

VALUES OF SOME MOLECULAR PARAMETERS†

Listed in order of decreasing $\Lambda^*$

| | $\varepsilon_0$ ($10^{-15}$ erg) | $r_0$ (Å) | $\Lambda^*$ | $P_0$ (atm) | $V_0$ (cm³/mole) | $T_0$ (°K) | $u_0$ (m/sec) |
|---|---|---|---|---|---|---|---|
| He | 1·41 | 2·87 | 2·64 | 84·5 | 10·06 | 10·2 | 146 |
| $H_2$ | 5·11 | 3·29 | 1·73 | 203 | 15·12 | 37·0 | 390 |
| $CH_4$ | 20·5 | 4·29 | 0·23 | 362 | 33·5 | 148 | 277 |
| $N_2$ | 13·2 | 4·17 | 0·22 | 254 | 31·3 | 96 | 169 |
| $O_2$ | 16·3 | 3·89 | 0·21 | 390 | 24·9 | 118 | 175 |
| A | 16·5 | 3·82 | 0·19 | 415 | 24·0 | 120 | 158 |
| $CCl_4$ | 45·1 | 6·61 | 0·03 | 219 | 122 | 320 | 133 |

† Based on values of $\varepsilon_0$ and $r_0$ for the 12 : 6 potential (**11**), taken from the tables of Hirschfelder, Curtiss, and Bird (1954, p. 1110).

de Boer's formulation of the law of corresponding states has a number of advantages over the more usual formulation in terms of the critical constants. The main advantage is that it reveals quantal phenomena which are obscured in the conventional treatment because the critical constants are themselves influenced by quantum effects. It has been applied to the $P$-$V$-$T$ behaviour of

dense gases (Hamann 1957, p. 44), of liquids (de Boer and Lunbeck 1948), and of solids (de Boer and Blaisse 1948; Salter 1954; Hamann 1957, pp. 49, 64), and to the thermal conductivities of dense gases (Owens and Thodos 1957) and of solids (Keyes 1959). We shall now apply it to the speed of sound in liquids.

The compressibility of a fluid has the dimensions of inverse pressure, so that it can be expressed in a reduced form by multiplying it by $P_0$, that is,

$$\varkappa_T^* = \varkappa_T P_0, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (17)$$

$$\varkappa_S^* = \varkappa_S P_0 = \varkappa_T P_0/\gamma. \quad \ldots\ldots\ldots\ldots\ldots\ldots (18)$$

It should be noted that whereas $\varkappa_T^*$ can be derived directly from the function $f_1$ (eqn. **15**), $\varkappa_S^*$ requires additional knowledge of the value of $\gamma$. Combining the relations (**2**), (**3**), and (**17**) we find that

$$u = \left(\frac{P_0 V_0}{M} \cdot \frac{\gamma V^*}{\varkappa_T^*}\right)^{\frac{1}{2}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots (19)$$

so that the appropriate molecular unit for the speed of sound is

$$u_0 = \left(\frac{P_0 V_0}{M}\right)^{\frac{1}{2}} = \left(\frac{N\varepsilon_0}{M}\right)^{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots\ldots (20)$$

The reduced value of $u$ is then

$$u^* = \frac{u}{u_0} = \left(\gamma \frac{V^*}{\varkappa_T^*}\right)^{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots (21)$$

Some values of $u_0$ are listed in the last column of Table 1.

In the general case of a liquid composed of complex polyatomic molecules, $\gamma$ is a function of $P^*$, $T^*$, $\Lambda^*$, and of the internal structure of the molecules. But in monatomic liquids and in diatomic liquids whose molecular rotation is substantially unexcited, it depends only upon $P^*$, $T^*$, and $\Lambda^*$ (or upon $V^*$, $T^*$, and $\Lambda^*$). It follows that the reduced speed of sound then has the general form

$$u^* = f_2(P^*, T^*, \Lambda^*), \quad \ldots\ldots\ldots\ldots\ldots (22)$$

where $f_2$ is a universal function for this class of liquids.

If the only pressure acting on the liquid is that of the atmosphere or of its own vapour, then at temperatures below the normal boiling point (at $T^* \approx 0{\cdot}8$) $P^*$ is very small and can be considered constant. Under these conditions $u^*$ depends only upon $T^*$ and $\Lambda^*$. If in addition the molecules are heavy, $\Lambda^*$ is small and $u^*$ is then uniquely determined by the reduced temperature $T^*$, that is,

$$u^* = f_3(T^*), \quad \ldots\ldots\ldots\ldots\ldots (23)$$

where $f_3$ is another universal function.

## III. Experimental Data

Unfortunately, there have been very few measurements of the speed of sound in the liquefied inert gases. There are some data for He over a rather limited temperature range (Findlay *et al.* 1939) and some for A (Liepmann 1939).

Itterbeek and his collaborators have made a wider range of measurements on liquid $H_2$ (Itterbeek and Verhaegen 1949*a*), $O_2$ and $N_2$ (Itterbeek, de Bock, and Verhaegen 1949), and $CH_4$ (Itterbeek and Verhaegen 1949*b*). There are also some results for $CCl_4$ (Landolt-Börnstein 1936).

These data have been plotted in a reduced form in Figure 1.

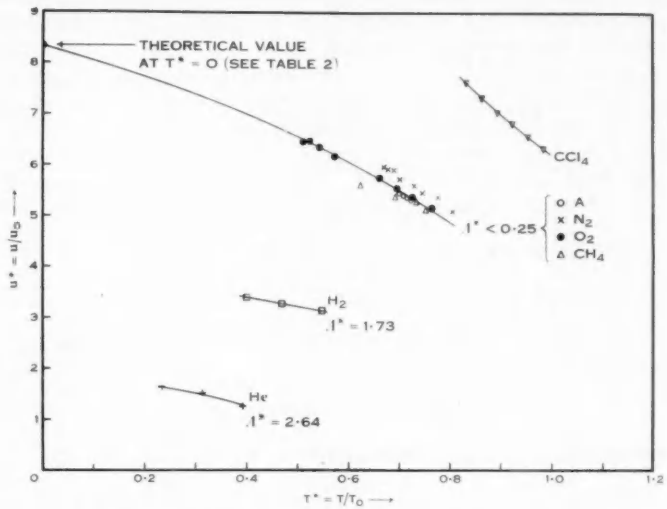


Fig. 1.—The reduced speed of sound in simple liquids at low pressures, plotted against the reduced temperature. The molecular units $u_0$ and $T_0$ are listed in Table 1.

## IV. Discussion

It is clear from Figure 1 that the more "classical" liquids A, $N_2$, $O_2$, and $CH_4$ conform quite well to the principle of corresponding states as expressed in equation (**23**). But $H_2$ and He deviate in proportion to their quantal parameters $\Lambda^*$. The reason for this is that the large zero-point energy of the light liquids inflates their volume (de Boer and Lunbeck 1948) and renders them much more compressible than the classical liquids. The increase in compressibility is greater than the increase in volume so that, by formula (**21**), $u^*$ is less than the classical value. $CCl_4$ deviates in the opposite direction for reasons which will be discussed later in this section.

It is possible to derive the functions $f_2$ and $f_3$ in a purely theoretical way from the Lennard-Jones and Devonshire (1937) model for liquids, applying a quantum correction for the lighter liquids (Hamann 1952). Mr. H. G. David is at present carrying out these calculations and the results will be published when they are complete. In the meantime it is worthwhile to consider the particularly simple case of a classical Lennard-Jones and Devonshire liquid at absolute zero.

In this theory the liquid is assumed to consist of a cubic close-packed lattice of molecules, having a reduced lattice energy

$$E^* = E/N\varepsilon_0 = A/V^{*\frac{1}{3}n} - B/V^{*\frac{1}{3}m}, \quad \ldots\ldots\ldots\ldots \quad (24)$$

where $E$ is the lattice energy per mole and $A$ and $B$ are lattice summations analogous to the Madelung constants in ionic crystals. $n$ and $m$ are the same exponents as those in the pair interaction energy (**10**). The reduced pressure and isothermal compressibility are then

$$P^* = -\frac{dE^*}{dV^*} = (nA/V^{*\frac{1}{3}n} - mB/V^{*\frac{1}{3}m})/3V^*, \quad \ldots\ldots\ldots\ldots\ldots \quad (25)$$

$$\varkappa_T^* = -\frac{1}{V^*}\frac{dV^*}{dP^*} = 9V^*/[n(n+3)A/V^{*\frac{1}{3}n} - m(m+3)B/V^{*\frac{1}{3}m}]. \quad \ldots\ldots\ldots\ldots\ldots \quad (26)$$

It is an experimental fact that $\gamma = 1$ at absolute zero, so that $\varkappa_S^* = \varkappa_T^*$ and

$$u^* = (V^*/\varkappa_T^*)^{\frac{1}{2}} = \tfrac{1}{3}[n(n+3)A/V^{*\frac{1}{3}n} - m(m+3)B/V^{*\frac{1}{3}m}]^{\frac{1}{2}}. \quad \ldots\ldots \quad (27)$$

These expressions can be greatly simplified if we consider only the interaction of nearest neighbours in the lattice. Then

$$A = 6m/(n-m), \quad B = 6n/(n-m), \quad \ldots\ldots\ldots\ldots \quad (28)$$

and if the system is at a low pressure, $P^* = 0$, $V^* = 1$, $E^* = -6$, and

$$\varkappa_T^* = \varkappa_S^* = 3/2nm, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (29)$$

$$u^* = (2nm/3)^{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (30)$$

The last of these relations is important because it shows that the reduced speed of sound increases with an increase in the product of the exponents $n$ and $m$. This may well explain the positive deviation of $CCl_4$ in Figure 1. Hamann and Lambert (1954) have suggested that the mutual potential energy between large globular molecules like $CCl_4$ is much closer to a 28 : 7 potential ($n = 28$, $m = 7$) than to the 12 : 6 potential which holds for small monatomic and diatomic molecules. The value of $u^*$ should be correspondingly larger.

TABLE 2

CALCULATED PROPERTIES OF A CUBIC CLOSE-PACKED LATTICE OF 12 : 6 MOLECULES FOR $T^* = 0$, $P^* = 0$, $\Lambda^* = 0$

| Property | Nearest Neighbours Only | Complete Lattice Summation | Property | Nearest Neighbours Only | Complete Lattice Summation |
|---|---|---|---|---|---|
| Lattice constant $A$ | 6 | 6·066 | Lattice volume $V^*$ | 1 | 0·916 |
| Lattice constant $B$ | 12 | 14·454 | Compressibility $\varkappa^*$ | 0·0208 | 0·0133 |
| Lattice energy $E^*$ | —6 | —8·61 | Speed of sound $u^*$ | 6·93 | 8·30 |

However, it is important to bear in mind that the relations (29) and (30) are only valid at absolute zero and for an idealized model. Bradley and Drury (1959) have recently applied a relation equivalent to (29) to the experimental compressibility of $CBr_4$ at 311 °K. They concluded from the apparent value of $nm$ that the molecules must obey a 9 : 6 potential rather than the 28 : 7 potential proposed by Hamann and Lambert (1954). This conclusion is quite unjustified because the compressibility decreases rapidly with decreasing temperature and at absolute zero it could easily be small enough to correspond with the 28 : 7 potential.

The simple theory presented above can be improved if particular values of $n$ and $m$ are selected. It is then possible to use the complete lattice summations for $A$ and $B$ which Lennard-Jones and Ingham (1925) have worked out. The results of this treatment are shown in Table 2 for a 12 : 6 potential. The value $u^*=8{\cdot}30$ has been plotted in Figure 1. It will be seen that the experimental data for the " classical " liquids extrapolate quite smoothly to this theoretical value at absolute zero.

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# THE THERMODYNAMIC PROPERTIES OF THE LOWER CHLORIDES OF TITANIUM

By R. B. Head*

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*Summary*

The free-energy values for titanium trichloride were determined to within 0·5 kcal per mole, using a " null " method with mercury and silver as reductants. Attempts to obtain free-energy values by means of the hydrogen reduction of titanium tetrachloride vapour failed to give reliable results owing to reactions between the trichloride and the glass ampoule. Thermodynamic data for titanium dichloride were obtained by vacuum disproportionation of the trichloride.

## I. Introduction

Some years ago, the only thermodynamic data for titanium tri- and dichlorides were estimates of the heats of formation and the free-energy functions. Many of these estimates were based on inaccurate values for the heat of formation of titanium tetrachloride. More recently, calorimetric determinations by Skinner and Ruehrwein (1952) and by Johnson, Nelson, and Prosen (1954) led to the more accurate figures $-191\cdot0\pm3$ and $-192\cdot9\pm0\cdot6$ kcal per mole respectively, and in the present paper the last value is adopted for $\Delta H_{298}$ $TiCl_4$. Brewer *et al.* (1950) estimated $\Delta H_{298}$ $TiCl_3$ as $-165$ kcal per mole, a figure only 5 kcal lower than the most recent values determined. Schäfer and Zeppernick (1953) obtained $-161\cdot6$ kcal per mole, which when corrected for $\Delta H_{298}TiCl_4$, yielded $-173\cdot1$ kcal per mole.

The following three methods were investigated to secure thermodynamic data for titanium trichloride:

(i) Reduction with hydrogen

$$2TiCl_4+H_2\rightleftharpoons 2TiCl_3+2HCl. \quad \ldots\ldots\ldots\ldots \quad (1)$$

(ii) Reduction with mercury

$$TiCl_4+Hg\rightleftharpoons \tfrac{1}{2}Hg_2Cl_2+TiCl_3. \quad \ldots\ldots\ldots\ldots \quad (2)$$

(iii) Reduction with silver

$$TiCl_4+Ag\rightleftharpoons AgCl+TiCl_3. \quad \ldots\ldots\ldots\ldots \quad (3)$$

The last two were discovered by Thorpe (1885), who found a reversal of reaction at about 250 °C for mercury.

A figure of $-114$ kcal per mole has been published by the National Bureau of Standards for the heat of formation of the dichloride. Another estimate by

* School of Metallurgy, University of Melbourne; present address: Division of Physical Chemistry, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

Brewer *et al.* (1950), when corrected for the $\Delta H_{298}TiCl_4$ figure yields $\Delta H_{298}TiCl_2 = -122$ kcal per mole. This was derived from data on the disproportion reaction studied by Fast (1938):

$$2TiCl_3 \rightleftharpoons TiCl_4 + TiCl_2, \quad \ldots\ldots\ldots\ldots\ldots\ (4)$$

which was used in the present study.

## II. Experimental Methods

### (*a*) *Hydrogen Reduction of Titanium Tetrachloride*

A quantity of commercial titanium tetrachloride was purified by refluxing over copper metal followed by several fractional distillations after McTaggart (1945) to yield a final portion boiling at 409·4 °C. Pyrex ampoules (0·2 ml), previously heated to softening point in a stream of dry pure hydrogen, were filled by distilling the purified tetrachloride into them. After sealing, the residual volume of hydrogen was less than 0·01 ml. These were weakened by a scratch, weighed and enclosed in 40 ml Pyrex ampoules filled with hydrogen after previous heating in a stream of hydrogen. The small ampoules were broken by shaking.

At temperatures below 550 °K the movement toward equilibrium was very slow but even here the glass was showing signs of attack, with gradual disappearance of solid trichloride. Whether the glass was borosilicate or one high in alumina a brown product formed. Both the rate of reduction and the attack on glass rose with temperature but by heating for various periods at 759 °K an estimate could be made of the equilibrium concentrations. The ampoules were quenched in a blast of nitrogen, opened under a solution of ferric ammonium sulphate swept by nitrogen, and the titanium trichloride determined using N/50 $K_2Cr_2O_7$ in the presence of phosphoric acid to ensure a sharp end-point using barium diphenylamine sulphonate as indicator. The weight of fragments from the 0·2 ml ampoules was subtracted to give the weight of titanium tetrachloride which was in roughly equimolar proportion to the hydrogen to ensure maximum sensitivity.

### (*b*) *Reduction of Titanium Tetrachloride with Mercury*

(i) *Four Condensed Phases.*—Thick-walled Pyrex ampoules of about 2 ml containing 0·2 g triple-distilled A.R. mercury were filled by distilling in the tetrachloride in a stream of nitrogen. Sufficient volume was left for expansion, the ampoules being under considerable internal pressure during the experiment. These ampoules were hung inside a hollow aluminium block of a vertical furnace controlled to within 1 °C. The temperature at the level of the mercury was measured by a movable thermocouple calibrated to $\pm 0{\cdot}5$ °C.

The critical temperature for which the free-energy change for reaction (2) is zero could be detected by means of a colour change due to the appearance or disappearance of the red trichloride on the surface of the mercury. The mercury was examined by dropping the ampoules from the furnace on Nichrome wires, the temperature being raised gradually until the red colour of the trichloride could no longer be observed. The equilibrium temperature was approached from both sides. Near this temperature the rate was slow so that 24 hr was allowed

for each trial temperature. Small amounts of titanium oxychlorides had no effect on the equilibrium temperature. This procedure confined the experimental errors to the temperature measurement.

(ii) *Three Condensed Phases, One Gas Phase.*—The mercury and a small weighed ampoule of titanium tetrachloride were sealed in an evacuated ($<10^{-3}$ mm Hg) ampoule of about 5 ml capacity. The reaction system is univariant as the equilibrium temperature depends on the pressure of the tetrachloride gas. From the volumes of the large ampoule, the mercury, and the internal ampoule, the pressure $P$ (assuming a perfect gas) could be calculated. The free-energy change at the critical equilibrium temperature is

$$\Delta G_R = -RT \ln P_{TiCl_4}. \quad \ldots\ldots\ldots\ldots\ldots \quad (5)$$

### (*c*) *Reduction of Titanium Tetrachloride with Silver*

The methods used were precisely those of Subsections (*b*) (i) and (*b*) (ii). The visual contrast was found to depend on the physical form of the silver, the best contrast being obtained from precipitated silver powder sintered for a few minutes at 600 °C. The reaction rate near the equilibrium temperature was rapid so that it was much more difficult to observe due to temperature changes when dropped from the furnace.

### (*d*) *Disproportionation to Produce Titanium Dichloride*

A suitable method of determining the free energy of formation of the dichloride is to obtain equilibrium pressures, which avoids handling an unstable substance. These were measured by a variation of the Hargreaves (1939) dew-point method in which the volume of a small quantity of liquid titanium tetrachloride was measured.

The rate of reaction for equation (4) is slow below 600 °K. It was necessary to use fused silica ampoules for higher temperatures. Each ampoule contained a platinum bucket supported at the upper end and terminated in a tube of 3 mm internal bore at the lower end. The ampoule was held in a steel block in a furnace, the tube projecting into an oil-bath. The temperature at the level of the bucket was measured with a calibrated thermocouple ($\pm 0{\cdot}5$ °C) and that of the oil-bath by a calibrated mercury-in-glass thermometer ($\pm 0{\cdot}1$ °C). About 15 g titanium trichloride was placed in the bucket.

Since it is difficult to remove the residual tetrachloride from the trichloride made by the Sherfey (1951) process, the ampoule containing its charge of trichloride was first swept out with purified hydrogen at about 350 °C, and was sealed off after cooling at a pressure of less than $10^{-3}$ mm Hg.

The liquid tetrachloride formed by disproportionation was allowed to collect in the end of the 3 mm tube, and the position of the meniscus was obtained by projecting it optically onto a scale. A volume change of less than a microlitre could be observed.

On initially heating the furnace to 700 °K, liquid tetrachloride collected quickly in the quartz stem immersed in the oil-bath. The temperature of the oil-bath was then increased until the quantity of titanium tetrachloride began to diminish. It was found possible to hold the position of the meniscus steady for

several minutes. At this temperature dew was observed to form just above the meniscus. The meniscus method was rather more sensitive than the dew-point method as a means of determining the equilibrium vapour pressure of the tetrachloride, since a change of less than 0·4 °C in the oil-bath temperature resulted in a slow, steady movement of the liquid level. At high temperatures the trichloride tended to sublime slowly into the stem.

## III. Experimental Results and Discussion

### (*a*) *Titanium Trichloride*

The equilibrium constant for the reaction of the tetrachloride with hydrogen was determined from

$$K_p=\frac{Vx^2\times 273}{22\cdot 4(a-x)^2(b-x)T},$$

where $T$ °K is the temperature for the reaction, $V$ (l.) the volume of the system, $2a$ moles of tetrachloride being initially present with $b$ moles of hydrogen. $2x$ moles is the yield of trichloride. Because of gradual disappearance of the trichloride during prolonged heating at 759 °K the value of $K_p$ was uncertain and can only be said to approach $1\cdot 4\times 10^{-3}$, giving the free-energy change for equation (**1**) as 10 kcal. Thermodynamic data used in this and the following sections were taken from the National Bureau of Standards Circular No. 500 and the Bureau of Mines Bulletins Nos. 383 and 476. The heat of formation of HCl was taken as −22·063 kcal per mole. The heat and entropy increments for tetrachloride were calculated with the aid of Kelley's equations in the form :

$$(\Delta C_p(\mathrm{TiCl_4})=C_p(\mathrm{TiCl_4})-C_p(\mathrm{Ti})-C_p(2\mathrm{Cl_2}))$$

from which

$$\Delta C_p(\mathrm{TiCl_4})_i=13\cdot 11-2\cdot 6\times 10^{-3}T+1\cdot 36\times 10^5T^{-2}.$$

The free energy of volatilization :

$$\Delta G_V=13{,}050+26\cdot 5T\log T-101\cdot 10T$$

based on $C_p=36\cdot 0$ agrees well with that calcula'ed from the vapour pressure equations of Schäfer and Zeppernick (1953), namely

$$\Delta G_V=13{,}200+26\cdot 5T\log T-101\cdot 4T.$$

Heat and entropy increments for the trichloride were estimated by comparison with $VCl_3$ using Kelley's equation for $C_p$ $VCl_3$.

The experimental data are shown in Table 1, and the calculated value for the free energy of titanium trichloride obtained from this reaction is shown in Table 2.

The four values for the free energy of formation of the trichloride obtained with metallic reductants are calculated using other data from the preceding section on tetrachloride and are also shown in Table 2. The heats of formation for $Hg_2Cl_2$ and AgCl are taken as −63·32 and −30·362 kcal per mole respectively. Two points due to Schäfer, Breuil, and Pfeffer (1954) have been included. These

were obtained for the reduction of the tetrachloride vapour by mercury using a dew-point method to determine the critical equilibrium vapour pressure. The free-energy curve for the trichloride is shown in Figure 1, and the consistency of the seven points is shown as a " third law " calculation in Table 3.

TABLE 1

RESULTS FOR THE REDUCTION OF TITANIUM TETRACHLORIDE WITH HYDROGEN AT 759 °K

| Experiment | Time of Heating (hr) | Seal Off Temperature (°C) | Weight (g) | | Log $K_p$ |
|---|---|---|---|---|---|
| | | | Tetrachloride | Trichloride | |
| 1 | 4 | 17 | 0·2481 | 0·00807 | —3·168 |
| 2 | 24 | 17 | 0·2007 | 0·00887 | —3·029 |
| 3 | 24 | 18 | 0·1654 | 0·00548 | —3·150 |
| 4 | 24 | 18 | 0·1783 | 0·00551 | —3·214 |
| 5 | 60 | 18 | 0·1933 | 0·00416 | —3·541 |
| 6 | 6 | 18 | 0·1406 | 0·00619 | —2·892 |
| 7 | 1·5 | 18 | 0·1767 | 0·00724 | —2·981 |

TABLE 2

CALCULATION OF THE MOLAL FREE-ENERGY VALUES FOR TITANIUM TRICHLORIDE

| Reduction by | Critical Equilibrium Temperature (°K) | $\Delta G$ $TiCl_4$ (kcal/mole) | $\Delta G_R$ (kcal/mole) | $\Delta G$ $TiCl_3$ (kcal/mole) |
|---|---|---|---|---|
| Hg in gas* | 411·0 | —171·5 | —1·762 | —150·6 |
| Hg in gas* | 426·0 | —170·8 | —1·196 | —149·6 |
| Hg in gas | 447·7 | —170·2 | —0·164 | —148·4 |
| Hg in liquid | 488·5 | —167·5 | — | —146·4 |
| Ag in gas | 570·0 | —166·7 | +1·807 | —142·1 |
| Ag in liquid | 642·0 | —160·6 | — | —138·7 |
| $H_2$+gas | 759·0 | —161·3 | +5·0 | (—132·7) |

* Schäfer, Breuil, and Pfeffer (1954).

Since the variation of entropy with temperature cannot be observed over the limited range of the experimental points, it has been assumed that the slope of the curve is equal to the entropy at the mean temperature of the reliable points. Taking this mean temperature as 530 °C, the following values are obtained :

$$\Delta S_{298}\ TiCl_3 = -53{\cdot}3 \text{ cal/deg/mole},$$
$$S^\circ\ TiCl_3 = 34{\cdot}0 \text{ cal/deg/mole},$$
$$\Delta H_{298}\ TiCl_3 = -172{\cdot}3 \pm 1 \text{ kcal/mole}.$$

If the various published values of $\Delta H_{298}$ $TiCl_4$ shown in Table 4 are considered it will be seen that the values of $\Delta H_{298}$ $TiCl_3$ calculated from the free energies determined in this work vary considerably. A comparison with a recent calorimetric determination of $\Delta H_{298}$ $TiCl_3$ shows that the result by Skinner and Ruehrwein (1952) is close to the calculated value based on the " best " values for $\Delta H_{298}$ $TiCl_4$.

### (*b*) *Titanium Dichloride*

The free energy for the reaction:

$$2TiCl_3 \rightleftharpoons TiCl_4 + TiCl_2$$

is given by

$$\Delta G_R = -RT \ln K_p,$$

where $-4 \cdot 575T \log P = 13{,}050 + 26 \cdot 5T \log T - 101 \cdot 10T$ (Kelley). The values of $\Delta G$ $TiCl_4$ were calculated in the same way as for the previous calculation on

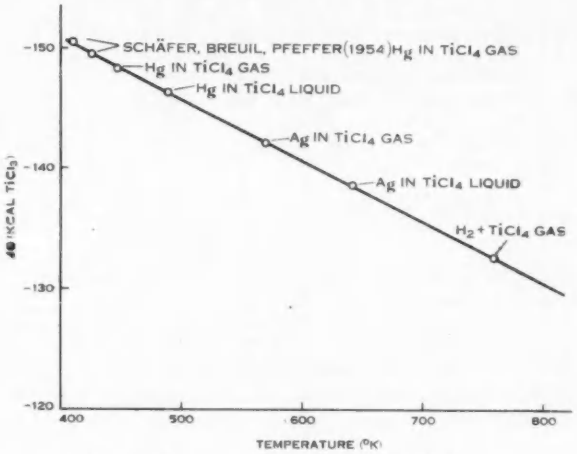


Fig. 1.—Free-energy curve for titanium trichloride.

the trichloride. Owing to the uncertainty in the values used for calculating the heat and entropy increments for the trichloride, its free-energy figures were obtained by extrapolation from the free-energy curve shown in Figure 1.

TABLE 3

"THIRD LAW" CALCULATION FOR TITANIUM TRICHLORIDE

Based on free-energy data obtained at various temperatures

| Temperature (°K) | $\Delta H_{298}$ $TiCl_3$ (kcal/mole) | Temperature (°K) | $\Delta H_{298}$ $TiCl_3$ (kcal/mole) |
|---|---|---|---|
| 411·0 | 172·8 | 570·0 | 172·0 |
| 426·0 | 172·5 | 642·0 | 172·0 |
| 447·0 | 172·4 | 759·0 | (171·4) |
| 488·5 | 172·3 | | |

Heat and entropy increments for the dichloride were estimated from Kelley's equations for $VCl_2$, $MnCl_2$, and $FeCl_2$.

TABLE 4

CALCULATION OF THE HEAT OF FORMATION OF TITANIUM TRICHLORIDE BASED ON VARIOUS PUBLISHED VALUES OF $\Delta H_{298}$ $TiCl_4$

| $\Delta H_{298}$ $TiCl_4$ (kcal/mole) | Source | $\Delta H_{298}$ $TiCl_3$ | |
|---|---|---|---|
| | | Calculated (kcal/mole) | Measured (kcal/mole) |
| −179·3 | Nat. Bur. Stand. (1952) | −158·7 | |
| −181·4 | Brewer *et al.* (1950) | −160·8 | |
| −183·0±2·5 | Kubaschewski and Evans (1951) | −162·4±3·7 | |
| −185·0 | Roth and Becker (1932) | −164·4 | |
| −186·0 | Munster and Ruppert (1953) | −165·4 | |
| −191·0±3* | Skinner and Ruehrwein (1952) | −170·4±4 | −170±3·8 heat of solution |
| −192·9±0·6* | Johnson, Nelson, and Prosen (1954) | −172·3±1 | Skinner and Ruehrwein (1952) |

* Calorimetric determination.

The calculations for $TiCl_2$ are shown in Table 5 and were carried out in the same manner as those previously described for $TiCl_3$, giving the following results.

$$\Delta S_{298}\ TiCl_2 = -37\cdot0 \text{ cal/deg/mole},$$
$$S^\circ\ TiCl_2 = 24\cdot0 \text{ cal/deg/mole},$$
$$\Delta H_{298}\ TiCl_2 = -122\cdot4 \pm 3 \text{ kcal/mole}.$$

TABLE 5

CALCULATION OF THE MOLAL FREE-ENERGY VALUES FOR TITANIUM DICHLORIDE

| Furnace Temp. (°K) | Stem Temp. (°K) | $\Delta G_R$ (kcal) | $\Delta G$ (kcal) | | | $\Delta H_{298}$ $TiCl_2$ (kcal) (Third Law) |
|---|---|---|---|---|---|---|
| | | | $TiCl_3$ | $TiCl_4$ | $TiCl_2$ | |
| 772·5 | 299·2 | +6·28 | −132·0 | −160·9 | −96·8 | −126·9 |
| 785·0 | 296·5 | +6·61 | −131·4 | −160·6 | −95·6 | −125·6 |
| 822·1 | 322·0 | +4·84 | −129·5 | −159·5 | −94·7 | −124·5 |
| 825·0 | 323·25 | +4·76 | −129·4 | −159·4 | −94·5 | −124·4 |
| 849·7 | 339·7 | +3·73 | −128·1 | −158·7 | −93·8 | −123·6 |
| 856·9 | 340·0 | +3·74 | −127·8 | −158·5 | −93·3 | −123·0 |
| 866·0 | 348·0 | +3·25 | −127·3 | −158·3 | −93·1 | −122·8 |
| 869·0 | 345·0 | +3·46 | −127·1 | −158·2 | −92·6 | −122·3 |
| 871·5 | 352·5 | +2·98 | −127·0 | −158·1 | −92·9 | −122·6 |
| 883·0 | 359·0 | +2·61 | −126·4 | −157·8 | −92·5 | −122·1 |
| 889·0 | 366·8 | +2·16 | −126·1 | −157·7 | −92·5 | −122·1 |
| 893·0 | 366·3 | +2·20 | −125·9 | −157·5 | −92·1 | −121·8 |
| 895·0 | 371·0 | +1·94 | −125·8 | −157·4 | −92·3 | −121·9 |
| 902·0 | 373·0 | +1·84 | −125·5 | −157·2 | −91·9 | −121·4 |
| 904·0 | 376·5 | +1·64 | −125·4 | −157·2 | −91·9 | −121·4 |
| 970·0 | 413·0 | −0·02 | −122·0 | −155·3 | −88·8 | (−118·0) |

From a plot of the entropies of the dichlorides of Cr, Mn, and Fe, $S^\circ$ $TiCl_2$ is estimated as $23 \cdot 5 \pm 1$ cal/deg/mole, which agrees well with the calculated value shown above.

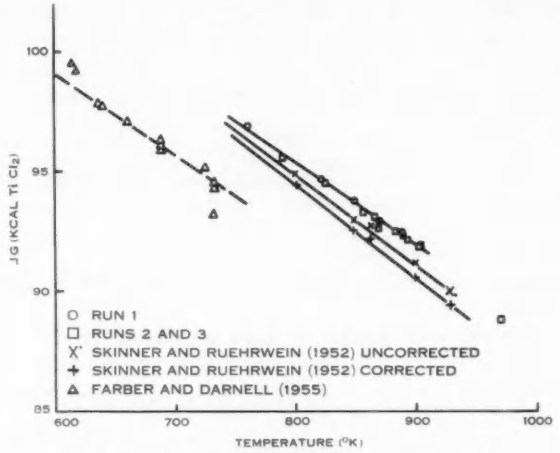


Fig. 2.—Free-energy curve for titanium dichloride.

While the work was progressing, Skinner and Ruehrwein (1952) performed a similar disproportionation using an alumina cup charged with the trichloride, and a heated mercury manometer which measured total pressure, and not the

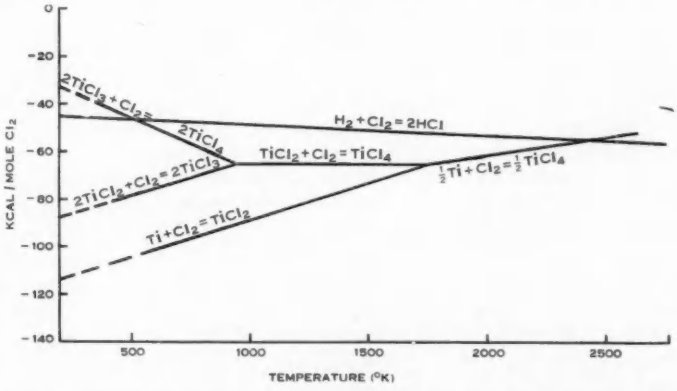


Fig. 3.—The relations between the chlorides of titanium. Standard free energy per mole of dichloride *v.* temperature.

partial pressure of tetrachloride. They corrected their results by subtracting an estimated partial pressure due to the trichloride vapour. These results have been recalculated, using the same figures for the free energy of tri- and tetra-

chlorides as were used in the above calculation. It is seen from the curves shown in Figure 2 that both sets of results lie within about 1·0 kcal of each other.

Farber and Darnell (1955) investigated the disproportionation of the trichloride by effusion from a molybdenum cell. Their results have been recalculated in a similar manner, and the resulting free-energy curve for the dichloride is shown also in Figure 2. The free energy is about 3 kcal/mole lower.

Skinner and Ruehrwein (1952) also investigated the reaction

$$2NaCl + Ti \rightleftharpoons 2Na + TiCl_2$$

by boiling sodium chloride in a titanium tube, and determining the amount of titanium present in the condensed salt. They give

$$\Delta H_{298}\ TiCl_2 = -118 \cdot 3 \pm 3 \text{ kcal/mole},$$

which is about 4·0 kcal lower than the figure obtained from the present investigation.

The relations between the three chlorides of titanium are shown in Figure 3 in the form of a free-energy diagram. All values are based on

$$\Delta H_{298}\ TiCl_4 = -192 \cdot 6 \pm 0 \cdot 6 \text{ kcal/mole}.$$

## IV. Acknowledgments

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# PREPARATION OF SOME AROMATIC *ISO*THIOCYANATES

By J. Cymerman Craig* and W. J. Neely*

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*Summary*

The preparation of some aromatic *iso*thiocyanates is described and their characteristic infra-red absorption bands near 2100 $cm^{-1}$ are reported.

## I. Introduction

The system (X=C=Y) is well known to show characteristic and intense infra-red absorption in the 2000–2200 $cm^{-1}$ region, for example, in allenes (C=C=C) and ketenes (C=C=O) (Bellamy 1958), keteneimines (C=C=N) (Stevens and French 1953, 1954), carbodi-imides (N=C=N) (Meakins and Moss 1957), and *iso*cyanates (N=C=O) (Davison 1953; Hoyer 1956).

Two alkyl *iso*thiocyanates have been reported (Kjaer and Rubinstein 1954; Kjaer and Jensen 1956) to exhibit a strong absorption band near 2150 $cm^{-1}$, and allyl (Carol and Ramsay 1953) and other aliphatic *iso*thiocyanates (Lieber, Rao, and Ramachandran 1959) have also been measured. It appeared desirable to locate the frequency characteristic of the system (N=C=S) in aromatic compounds, as an aid to the identification of *iso*thiocyanates in general. While this work was in progress, Caldow and Thompson (1958) reported the spectra of a number of substituted phenyl*iso*thiocyanates in solution.

## II. Preparation of Compounds

*p*-Chloro- (71 per cent. yield) and *p*-fluorophenyl*iso*thiocyanate (44 per cent. yield) were readily obtained using the method of Baxter *et al.* (1956). *p*-Methoxyphenylthiourea, prepared either by an adaptation of the excellent method of Frank and Smith (1948) or by that of de Clermont (1876), had very low solubility (0·5 per cent.) in boiling chlorobenzene, and *p*-methoxyphenyl*iso*thiocyanate could only be prepared (44 per cent. yield) in dilute solution in that solvent. *p-tert.*-Butylphenylthiourea, obtained in 81 per cent. yield, showed normal solubility in chlorobenzene (12·5 per cent.) and readily afforded 70 per cent. of *p-tert.*-butylphenyl*iso*thiocyanate.

*p*-Acetophenyl*iso*thiocyanate proved unexpectedly difficult to obtain. The corresponding thiourea was almost insoluble (0·2 per cent.) in chlorobenzene, and was impossible to convert to the *iso*thiocyanate using that solvent, while reaction using acetophenone as solvent yielded only 6 per cent. of the desired compound. Treatment of di-*p*-acetophenylthiourea with acetic anhydride (Crippa and Caracci 1940) gave quantitative conversion into the *iso*thiocyanate; however, this symmetrical dithiourea was only obtainable in 10 per cent. yield by reaction of the arylamine with carbon disulphide. Attention was therefore

* Organic Chemistry Department, University of Sydney.

directed to the decomposition of an unsymmetrically-disubstituted thiourea in acid medium, since it has been observed (Chattaway, Hardy, and Watts 1924) that the more strongly basic portion is isolated as the amine, the more weakly basic half of the molecule being converted to the *iso*thiocyanate. While *N*-*p*-acetophenyl-*N'*-phenylthiourea proved resistant to the action of 18N sulphuric acid, fission took place quantitatively with acetic anhydride. The

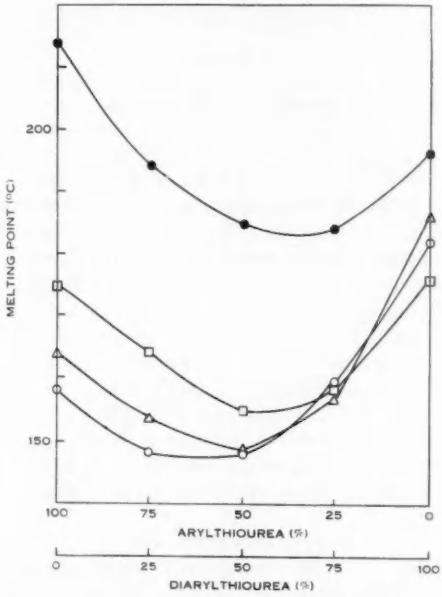


Fig. 1.—Melting point–composition diagram for mixtures of aryl- and diarylthioureas.
● Mono- and di-*p*-acetophenylthiourea.
□ Mono- and di-*p*-chlorophenylthiourea.
△ Mono- and di-*p*-fluorophenylthiourea.
○ Mono- and di-*p*-*tert*.-butylphenylthiourea.

products were found to consist of equal proportions of phenyl- and *p*-acetophenyl-*iso*thiocyanate on the one hand, and of acetanilide and *p*-acetoacetanilide on the other hand, indicating that, contrary to expectation, the decomposition had proceeded equally along both the possible pathways.

Due to the very closely similar, and frequently identical, melting points and solubilities of the arylthioureas and diarylthioureas, mixtures of these were analysed from melting point–composition curves and mixed melting points with the pure components and standard mixtures. Some typical melting point–composition plots are given in Figure 1.

The preparation of arylthioureas by heating an arylamine hydrochloride with ammonium thiocyanate is normally carried out in aqueous solution (cf.

Schroeder 1955) but chlorobenzene has also been recommended as solvent for this reaction (Passing 1939). The yields of arylthioureas obtained by Passing's modification are frequently erratic or low (e.g. Kurzer and Sanderson 1957) and it appeared probable that the use of chlorobenzene as solvent in this reaction was accompanied by fission of some of the thiourea when formed, to give the corresponding *iso*thiocyanate which, because of its high solubility in chlorobenzene, would not be detected by the normal methods of working-up the thiourea. Thus Kurzer and Sanderson (1957) report the preparation of 2,4,6-tribromophenylthiourea in 65–75 per cent. yield by Frank and Smith's (1948) method, whereas Passing's (1939) modification gave 34–41 per cent. of crude product. It is noteworthy that 5 mol of ammonium thiocyanate were required, the use of a smaller excess (2 mol) reducing the yield to 7–10 per cent. This observation may be explained by the reaction of any *iso*thiocyanate formed, with free ammonia (always present when ammonium thiocyanate is heated) to reform the thiourea, whereas a reduction in the amount of ammonium salt present would lessen this tendency.

The preparation of *p*-chlorophenylthiourea by reaction of *p*-chloroaniline hydrochloride with 5 mol of ammonium thiocyanate in chlorobenzene was therefore examined. When the concentration of the arylamine salt in chlorobenzene was 3·3 per cent. by weight (the solubility of the thiourea in boiling chlorobenzene), the results after 4·5, 8, and 13·5 hr showed in each case that approximately a 10 per cent. yield of arylthiourea, and a 33 per cent. yield of diarylthiourea, had been formed, and *c.* 12 per cent. of the *iso*thiocyanate was also isolated. An increase in the concentration of arylamine hydrochloride in chlorobenzene to 11 and to 37 per cent. by weight respectively, gave (after an 8-hr period in each case), a yield of arylthiourea of 14 and 23 per cent., the accompanying yields of diarylthiourea being 25 and 13 per cent. respectively. In each case appreciable quantities of *iso*thiocyanate were again isolated: 28 and 18 per cent. yields respectively.

The fact that the most concentrated reaction mixture gave the highest proportion of arylthiourea agrees with our findings that only that amount of the thiourea which is in solution in chlorobenzene will react to give ammonia and the *iso*thiocyanate. It is noteworthy that Kurzer and Sanderson's (1957) reaction mixture contained a 35 per cent. concentration of tribromoaniline hydrochloride in chlorobenzene, whereas the solubility of tribromophenylthiourea in chlorobenzene is probably less than 1 per cent. In our case, using *p*-chloroaniline, the crude product was seen to be a mixture of aryl- and diarylthiourea; the formation of the diarylthiourea must involve the prior hydrolysis (or ammonolysis) of some of the arylamine salt to give the free base, followed by reaction with the *iso*thiocyanate present.

It therefore appears that the reaction of arylamine hydrochlorides with an inorganic thiocyanate, to give the arylthiourea, is preferably carried out in aqueous medium; the use of chlorobenzene for this reaction is not to be recommended and results in the formation, by some decomposition of the thiourea formed, of the *iso*thiocyanate which then reacts with any free arylamine, present through hydrolysis of the arylamine salt, forming the diarylthiourea.

## III. Infra-Red Absorption Spectra

The infra-red absorption of the compounds has been measured by Dr. N. S. Ham and Dr. J. B. Willis, who have made a detailed analysis of the spectra (Ham and Willis 1960).

All *iso*thiocyanates exhibit a very intense pair of absorption bands in the 2100 $cm^{-1}$ region, characteristic of the antisymmetrical (N=C=S) vibration. The frequencies (liquids measured as capillary films, and solids as Nujol mulls) fall into the ranges 2050–2140 (vs) and 2170–2220 $cm^{-1}$ (ms to vs) and afford a useful means of identification of this group.

## IV. Experimental

(*a*) *Aryl* iso*Thiocyanates*.—The following were purified by sublimation *in vacuo*: *p*-Bromophenyl-, m.p. 57·5 °C; *p*-diphenylyl-, m.p. 66 °C; α-naphthyl-, m.p. 55·5 °C; β-naphthyl-, m.p. 59 °C; 9-phenanthryl-, m.p. 103·5–104·5 °C; 3-pyrenyl*iso*thiocyanate, m.p. 126–126·5 °C.

(*b*) p-*Chlorophenylisothiocyanate*.—*p*-Chlorophenylthiourea (6·0 g) and chlorobenzene (100 ml) were refluxed at 150 °C for 8 hr. Removal of chlorobenzene by distillation *in vacuo* and extraction of the residue with boiling hexane (4 × 50 ml) gave *p*-chlorophenyl*iso*thiocyanate (3·9 g, 71%), m.p. 46 °C. Dyson (1926) gives m.p. 44–45 °C. From a melting point–composition curve, the residue insoluble in hexane was found to be 40% *p*-chlorophenylthiourea and 60% di-*p*-chlorophenylthiourea.

(*c*) *Di*-p-*chlorophenylthiourea*.—Refluxing equal weights of *p*-chloroaniline and *p*-chlorophenyl*iso*thiocyanate in benzene for 30 min gave di-*p*-chlorophenylthiourea (96%), m.p. 176 °C. Dyson, George, and Hunter (1926) give m.p. 176 °C.

(*d*) p-*Methoxyphenylthiourea*.—Prepared by an adaptation of the method of Frank and Smith (1948) from *p*-anisidine (24·8 g), the thiourea (32·4 g, 88%) had m.p. 209 °C. Dyson and George (1924) give m.p. 210–211 °C. Its solubility in boiling chlorobenzene and boiling xylene was 0·5%.

(*e*) A solution of *p*-anisidine (49·2 g) in hydrochloric acid (1·5N) was refluxed for 9 hr with excess of ammonium thiocyanate, precipitated solid being removed at 3-hr intervals. The total yield of the thiourea was 59·3 g (83%), m.p. 209 °C.

(*f*) p-*Methoxyphenylisothiocyanate*.—*p*-Methoxyphenylthiourea (5·5 g) and chlorobenzene (250 ml) were refluxed for 8 hr. Working-up as described gave the *iso*thiocyanate (2·2 g; 44%), b.p. 103–104 °C/1·1 mm, 110 °C/2 mm, $n_D^{25}$ 1·6453, which solidified to white crystals, m.p. 21–22 °C. Dyson and George (1924) give b.p. 280–281 °C/760 mm; Coghill and Johnson (1925) give m.p. 18 °C. The hexane-insoluble residue (2·7 g) consisted of 88% *p*-methoxyphenylthiourea (44% recovery) and 12% di-*p*-methoxyphenylthiourea. The use of higher concentrations of *p*-methoxyphenylthiourea in chlorobenzene gave substantial recoveries of pure starting material.

(*g*) *Di*-p-*methoxyphenylthiourea*.—*p*-Anisidine was refluxed in benzene with excess of carbon disulphide for 4 hr, affording a quantitative yield of di-*p*-methoxyphenylthiourea, m.p. 190 °C. Dyson, George, and Hunter (1927) give m.p. 187 °C.

(*h*) p-*Fluorophenylisothiocyanate*.—*p*-Fluorophenylthiourea (9·5 g) in chlorobenzene (150 ml) was refluxed for 10 hr. Working-up as described above gave the *iso*thiocyanate (3·75 g; 44%), b.p. 60 °C/1·3 mm, 66 °C/1·8 mm, $n_D^{25}$ 1·6178, which solidified to crystals, m.p. 23·5–25·5 °C. Browne and Dyson (1931) give b.p. 228 °C/760 mm, m.p. 12 °C. The hexane-insoluble residue (2·1 g) consisted of 25% of starting material and 75% of di-*p*-fluorophenylthiourea.

(*i*) *Di*-p-*fluorophenylthiourea*.—Prepared from the amine and excess of carbon disulphide in boiling benzene solution (6 hr), this (90% yield) had m.p. 186–187 °C. Browne and Dyson (1931) give m.p. 145 °C; Lubs and Fox (1937) report m.p. 186·5–188 °C.

(*j*) p-tert.-*Butylphenylthiourea*.—Prepared by an adaptation of Frank and Smith's (1948) method from *p-tert.*-butylaniline (37·3 g) the *thiourea* (42 g, 81% yield) crystallized from benzene as plates, m.p. 158 °C (Found: C, 63·7; H, 7·7; S, 15·4%. Calc. for $C_{11}H_{15}NS$: C, 63·5; H, 7·7; S, 15·4%). Its solubility in boiling chlorobenzene was 12·5%.

(*k*) p-tert.-*Butylphenylisothiocyanate.*—*p-tert.*-Butylphenylthiourea (10·4 g) and chlorobenzene (100 ml) were refluxed for 10 hr. Removal of chlorobenzene *in vacuo* and extraction of the residue with boiling hexane (7×40 ml) gave the *iso*thiocyanate (6·7 g, 70%), b.p. 116 °C/1·8 mm, 105 °C/1·3 mm, solidifying to crystals, m.p. 40 °C. Pahl (1884) gives b.p. 277 °C/760 mm, m.p. 42 °C. The hexane-insoluble residue (1 g) consisted of 5% recovered starting material and 95% of di-*p-tert.*-butylphenylthiourea.

(*l*) *Di*-p-tert.-*butylphenylthiourea.*—(i) Reaction of *p-tert.*-butylaniline and excess of carbon disulphide in boiling benzene (5 hr) afforded the bisthiourea (76%) as needles, m.p. 182 °C, from benzene. (ii) Prepared from equal weights of *p-tert.*-butylaniline and *p-tert.*-butylphenyl*iso*thiocyanate in boiling benzene, the bisthiourea (88%) crystallized from benzene as needles, m.p. 182 °C, undepressed on admixture with the material prepared in (i) (Found : C, 73·9 ; H, 8·3%. Calc. for $C_{21}H_{28}N_2S$ : C, 74·1 ; H, 8·3%). Pahl (1884) reports m.p. 192·5 °C for this substance.

(*m*) p-*Acetophenylthiourea.*—This was prepared by an adaptation of Frank and Smith's (1948) method from *p*-aminoacetophenone (13·5 g). The benzoyl derivative, precipitated by pouring into water, was hydrolysed by boiling with sodium hydroxide solution (12%) for 6 min, suspended material was removed by filtration, and the product obtained by acidification of the filtrate followed by addition of sufficient ammonia to redissolve benzoic acid. The thiourea (11·6 g, 60%) crystallized from alcohol as needles, m.p. 213–214 °C. Dyson, George, and Hunter (1926) give m.p. 215 °C. Its solubility in boiling chlorobenzene was only 0·2%.

(*n*) p-*Acetophenylisothiocyanate.*—(i) Refluxing *p*-acetophenylthiourea and chlorobenzene for 11·5 hr gave only recovered starting material. (ii) Refluxing *p*-acetophenylthiourea in acetophenone, in which the thiourea is readily soluble, gave only 6% of the *iso*thiocyanate, the residue being unchanged starting material. (iii) A solution of di-*p*-acetophenylthiourea (0·93 g) in acetic anhydride was refluxed for 25 min and the solvent removed *in vacuo*. Extraction of the residue with boiling hexane gave 0·52 g (100%) *p*-acetophenyl*iso*thiocyanate, m.p. 73 °C. Dyson, George, and Hunter (1926) give m.p. 76 °C. The hexane-insoluble residue (0·45 g) was *p*-aceto-acetanilide, m.p. and mixed m.p. 165–167 °C.

(*o*) N-p-*Acetophenyl*-N′-*phenylthiourea.*—A solution of *p*-aminoacetophenone (13·5 g ; 0·1 mole) and phenyl*iso*thiocyanate (13·5 g ; 0·1 mole) in boiling benzene set solid after 30 min. After a further 30 min boiling, the product (24·5 g, m.p. 135–155 °C) was collected and extracted with boiling acetone. The acetone-soluble portion (18·5 g, 70%) crystallized as plates, m.p. 157 °C, which gave a positive test with a solution of 2,4-dinitrophenylhydrazine and depressed the m.p. of diphenylthiourea. It was therefore the desired N-p-*acetophenyl*-N′-*phenylthiourea* (Found : C, 66·3 ; H, 5·3 ; S, 11·5%. Calc. for $C_{15}H_{14}N_2OS$ : C, 66·7 ; H, 5·2 ; S, 11·9%). The acetone-insoluble portion (2·1 g, 17%) was di-*p*-acetophenylthiourea, m.p. and mixed m.p. 195–197 °C.

(*p*) *Di*-p-*acetophenylthiourea.*—(i) Refluxing *p*-aminoacetophenone with excess of carbon disulphide in benzene for 10 hr gave only unchanged amine.

(ii) When the same reactants were refluxed in ethanol for 12 hr extraction with cold hydrochloric acid (3N) of the residue after removal of ethanol left di-*p*-acetophenylthiourea (10%), m.p. 197 °C. The hydrochloric acid solution on basification gave unchanged amine (87%). The yield in this experiment was not enhanced by carrying out the reaction at 100 °C in an autoclave (64 atm) for 9 hr.

(iii) Reaction of *p*-aminoacetophenone and *p*-acetophenyl*iso*thiocyanate in boiling benzene gave di-*p*-acetophenylthiourea (80%), m.p. and mixed m.p. 197 °C. Dyson, George, and Hunter (1926) give m.p. 198 °C.

(*q*) *Decomposition of* N-p-*Acetophenyl*-N′-*phenylthiourea.*—(i) Attempted decomposition of *N*-*p*-acetophenyl-*N′*-phenylthiourea (2·7 g) with sulphuric acid (25 g ; 18N) and superheated steam by an adaptation of the method of Chattaway, Hardy, and Watts (1924) gave only 0·2 g of a steam-volatile product, shown to be a mixture of *p*-acetophenyl- and phenyl*iso*thiocyanates. The residue in the sulphuric acid medium was unchanged starting material.

(ii) A solution of *N*-*p*-acetophenyl-*N′*-phenylthiourea (2·7 g) in acetic anhydride was refluxed for 30 min and the solvent removed *in vacuo*. Extraction with boiling hexane gave a viscous

liquid (1·8 g) which was separated into *p*-acetophenyl*iso*thiocyanate (0·85 g, 48%), m.p. 73 °C, insoluble in cold hexane, and phenyl*iso*thiocyanate (0·7 g; 52%), identified by boiling with excess of aniline, removal of free aniline with dilute hydrochloric acid, and isolation of diphenylthiourea (m.p. and mixed m.p.).

The residue (1·6 g) from the hexane extraction of the reaction mixture was recrystallized from boiling water, giving *p*-acetoacetanilide (1 g, 56%), m.p. and mixed m.p. 171 °C. The aqueous mother liquors contained acetanilide (0·6 g, 44%), m.p. and mixed m.p. 110–112 °C.

(*r*) *Reaction of* p-*Chloroaniline Hydrochloride and Ammonium Thiocyanate.*—*p*-Chloroaniline hydrochloride (33 g; 0·2 mole, dried at 80 °C) and ammonium thiocyanate (15·2 g; 0·2 mole, dried similarly) were refluxed in dry chlorobenzene (90 ml) for 8 hr. Working-up as described previously gave *p*-chlorophenyl*iso*thiocyanate (6 g, 18%), and a mixture (16·7 g) consisting of *p*-chlorophenylthiourea (8·7 g, 23%) and di-*p*-chlorophenylthiourea (8 g, 13%).

## V. Acknowledgments

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# SYNTHESIS OF TWO GEOMETRICAL ISOMERS OF HEXADECA-2,4,8,10-TETRAENOIC ACID AND THEIR *ISO*BUTYLAMIDES

By A. Meisters* and P. C. Wailes*

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*Summary*

Two of the stereoisomers of hexadeca-2,4,8,10-tetraenoic acid, namely the *trans*-2,*trans*-4,*trans*-8,*cis*-10-, and the *trans*-2,*trans*-4,*cis*-8,*cis*-10-isomers, have been synthesized. The insecticidal activities of their *iso*butylamides and those of the intermediate acetylenic acids towards the housefly, *Musca domestica*, have been examined. One of these acetylenic *iso*butylamides, namely N-*iso*butylhexadeca-*trans*-2,*trans*-4-diene-8,10-diynamide, is the $C_{20}$ analogue of the naturally occurring $C_{18}$ acetylene " anacyclin ".

## I. Introduction

Our previous papers have described the synthesis of hexadeca-2,4-dienoic acids (Wailes 1959) and 5,9-dimethyldeca-2,4,8-trienoic acids (Meisters and Wailes 1960). Among the *iso*butylamides of all these acids only one gave a measurable insecticidal activity. This was that derived from the *trans*-2,*trans*-4-isomer of 5,9-dimethyldeca-2,4,8-trienoic acid, and even here the activity was rather weak. In continuation of the search to define the structural features needed in an *iso*butylamide of high toxicity to insects, two amides derived from $C_{16}$ tetraenoic acids have been prepared in which the unsaturated units are separated by a dimethylene group. Their structures thus simulate the systems believed to confer highly toxic properties on two naturally occurring *iso*butylamides: affinin (I, $n=2$) and *neo*herculin (I, $n=3$). Both acids (II) now prepared are *trans*-2,*trans*-4-dienoic acids, but in one the unsaturation beyond the dimethylene group is a conjugated *cis*-*cis*-diene and in the other a conjugated *cis*-*trans*-diene unit.

$$CH_3.(CH{=}CH)_n.CH_2CH_2.CH{=}CH.CONHCH_2CH(CH_3)_2,$$
(I)

$$C_5H_{11}.\underset{c.}{CH}{=}\underset{c.}{CH}.\underset{c.\text{ or }t.}{CH}{=}CH.CH_2CH_2.\underset{t.}{CH}{=}\underset{t.}{CH}.CH{=}CH.COOH.$$
(II)

Although no definite relationship between activity and geometrical isomerism has yet been determined, these arrangements seemed most likely to produce activity and have led to amides with good knockdown properties and with toxicities approaching those of natural affinin.

* Organic Chemistry Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

## II. Discussion of Syntheses

### (*a*) *Hexadeca*-trans-*2*,trans-*4*,cis-*8*,cis-*10-tetraenoic Acid*

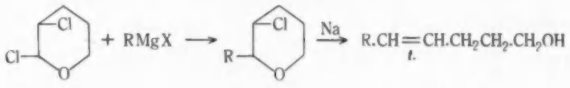
1-Bromohept-1-yne (III), prepared readily by the action of hypobromite on hept-1-yne (Straus, Kollek, and Heyn 1930), was coupled with pent-4-yn-1-ol (IV) (Jones, Eglinton, and Whiting 1953) in alcohol containing *iso*butylamine, a trace of cuprous chloride, and hydroxylamine hydrochloride (Chodkiewicz 1957 ; Bohlmann and Herbst 1958) to give dodeca-4,6-diyn-1-ol (V) in 60 per cent.


yield. Attempts to oxidize V to the corresponding aldehyde with *tert.*-butyl chromate and also with manganese dioxide in hot pyridine or water met with limited success. Yields of aldehyde were low and the alcohol was not always recoverable. It is probable that oxidation of the tosylate with dimethylsulphoxide, details of which were published after completion of this work (Kornblum, Jones, and Anderson 1959), would have produced the desired aldehyde. Instead dodeca-4,6-diyn-1-ol was oxidized to dodeca-4,6-diynoic acid (VI) with chromic acid and the dimethylamide of this acetylenic acid was reduced

to the aldehyde. The oxidation step produced considerable quantities of the ester from the diynoic acid and the diynol. Hydrolysis of this ester and reoxidation of the alcohol to acid and ester gave an 80–85 per cent. yield of acidic material and a 65 per cent. yield of recrystallized pure acid. Reduction of the dimethylamide of VI with lithium diethoxyaluminohydride (Brown and Tsukamoto 1959) gave dodeca-4,6-diynal (VII). Condensation of VII with 4-methoxybut-3-en-1-ynyl magnesium bromide (VIII) (Marshall and Whiting 1956) followed by treatment with ethanol to decompose the magnesium bromide complex gave the acetylenic carbinol (IX), the triple bond of which was then reduced to the *trans*-double bond by lithium aluminium hydride. Finally rearrangement of X with dilute acid produced hexadeca-*trans*-2,*trans*-4-diene-8,10-diynal (XI), which was oxidized to the corresponding acid (XII) with silver oxide. The *iso*butylamide of XII is an analogue of the naturally occurring acetylene anacyclin (Crombie 1955; Bohlmann and Inhoffen 1956; Crombie and Manzoor-i-Khuda 1957), in which the end alkyl group is *n*-propyl. Partial hydrogenation over Lindlar's catalyst (Lindlar 1952) gave, after five crystallizations, hexadeca-*trans*-2,*trans*-4,*cis*-8,*cis*-10-tetraenoic acid (XIII).

(*b*) *Hexadeca*-trans-*2*,trans-*4*,trans-*8*,cis-*10-tetraenoic Acid*

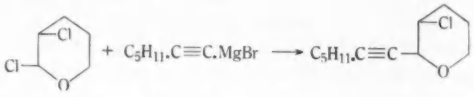
2,3-Dichlorotetrahydropyran (XIV) reacts with an alkyl magnesium bromide to give a 2-alkyl-3-chlorotetrahydropyran (XV). The latter, in the manner of β-halogeno-ethers, is split by an electropositive metal to give an olefinic alcohol (XVI) (Riobé 1950, and references therein; Crombie *et al.* 1956). Although both *cis*- and *trans*-halogeno-ethers (XV) are formed, the olefin resulting from fission is exclusively *trans* (Crombie and Harper 1950*a*, 1950*b*).



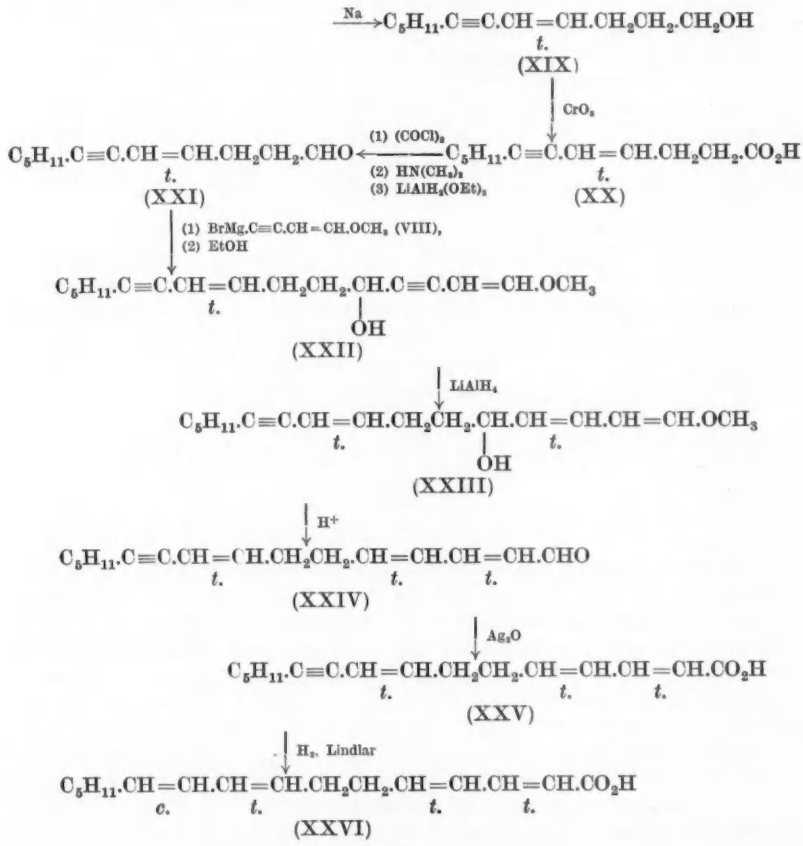

(XIV) (XV) (XVI)

The use of an alkynyl instead of an alkyl magnesium bromide was already under way in this laboratory before the work of Riobé (1953) was noticed. Although the details reported in Section V may differ in some respects from those of Riobé the overall result was the same. Hept-1-ynyl magnesium bromide (XVII) and dichlorotetrahydropyran readily formed a mixture of *cis*- and *trans*-2-heptynyl-3-chlorotetrahydropyran (XVIII). As with the alkyl compound, treatment with powdered sodium produced an apparently uniform product identified as dodeca-*trans*-4-en-6-yn-1-ol (XIX). Conversion to the aldehyde by way of the acid did not proceed as readily as with diynol (V). Oxidation with chromic acid led to more by-products and although a high yield of acidic material was obtained following the procedure described for the diynoic acid (VI), not all could be crystallized. The dimethylamide of the purified acid was reduced with lithium diethoxyaluminohydride to dodeca-*trans*-4-en-6-ynal (XXI) which was then subjected to the chain extension method with methoxybutenyny magnesium bromide (VIII) giving hexadeca-*trans*-2,*trans*-4,*trans*-8-trien-10-ynal (XXIV). Without isolation XXIV was oxidized with silver oxide to hexadeca-

*trans*-2,*trans*-4,*trans*-8-trien-10-ynoic acid (XXV) which was finally partially hydrogenated over Lindlar's catalyst to hexadeca-*trans*-2,*trans*-4,*trans*-8,*cis*-10-tetraenoic acid (XXVI).



(XIV) (XVII) (XVIII)




## III. Physical and Optical Properties

The tetraenoic acids and their acetylenic precursors described here are colourless crystalline solids which polymerize rapidly in the crystalline state. The tetraenoic acids polymerized faster than the acetylenic acids among which

TABLE 1

PHYSICAL AND SPECTRAL DATA OF COMPOUNDS

St, stretching, Be, bending vibration; s, strong, m, medium, w, weak band, sh, shoulder

| Compound | Melting Point (°C) | Ultraviolet Data* | | Infra-Red Data† | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\lambda_{max.}$ (mμ) | ε | C=O(St) | C=C(St) | H—C=(Be) *t.* | C=C.COX *t.* | N—H | C≡C(St) |
| **Acids** | | | | | | | | | |
| $C_5H_{11}.(C\equiv C)_2.CH_2CH_2.CO_2H$ | 66·5–68·5 | 213·5<br>224·5<br>238<br>252·5 | 500<br>490<br>400<br>230 | 1715s<br>1702sh | — | — | — | — | 2260w<br>2155w |
| $C_5H_{11}.(C\equiv C)_2.CH_2CH_2.(CH=CH)_2.CO_2H$ *t.* | 141–142 | 255·5 | 28,900 | 1686s<br>1674s | 1634m<br>1615m | 1006m<br>958w | 880w | — | |
| $C_5H_{11}.(CH=CH)_2.CH_2CH_2.(CH=CH)_2.CO_2H$ *c.* *t.* | 68·5–70·5 | 236<br>254·5 | 30,000<br>30,700 | 1688s<br>1676sh | 1634m<br>1614m | 1000m<br>935w | 876w | — | — |
| $C_5H_{11}.C\equiv C.CH=CH.CH_2CH_2.CO_2H$ *t.* | 61–62 | 228·5<br>235§ | 16,100<br>13,900 | 1710s<br>1695sh | — | 958s | — | — | 2225w |
| $C_5H_{11}.C\equiv C.CH=CH.CH_2CH_2.(CH=CH)_2.CO_2H$ *t.* *t.* | 119·5–120 | 235§<br>258 | 21,500<br>31,700 | 1695s<br>1680s | 1640m<br>1622m | 1007m<br>963m | 875m | — | 2230v.w |
| $C_5H_{11}.CH=CH.CH=CH.CH_2CH_2.(CH=CH)_2.CO_2H$ *c.* *t.* *t.* | 60–62·5 | 235·5<br>257 | 31,200<br>33,200 | 1692sh<br>1684s | 1638m<br>1618m | 1002m<br>984w<br>944w | 875w | — | — |
| ***iso*Butylamides** | | | | | | | | | |
| $C_5H_{11}.(C\equiv C)_2.CH_2CH_2.(CH=CH)_2.CONHBu^i$ *t.* | 114·5–115 | 258·5 | 36,000 | 1640s‡ | 1668s<br>1620s | 995s<br>950w | 860w | 3480w<br>3340w<br>1510s | 2260v.w<br>2160v.w |
| $C_5H_{11}.(CH=CH)_2.CH_2CH_2.(CH=CH)_2.CONHBu^i$ *c.* *t.* | 70·5–73·5 | 236·5<br>257 | 32,800<br>36,800 | 1630s | 1660s<br>1620s | 996s<br>940w | 872w | 3350s<br>1560s | — |
| $C_5H_{11}.C\equiv C.CH=CH.CH_2CH_2.(CH=CH)_2.CONHBu^i$ *t.* *t.* | 114–116 | 236§<br>258·5 | 25,800<br>37,100 | 1632s | 1662s<br>1618s | 998s<br>958sh<br>954m | 870w | 3350s<br>1552m | |
| $C_5H_{11}.CH=CH.CH=CH.CH_2CH_2.(CH=CH)_2.CONHBu^i$ *c.* *t.* *t.* | 68–69 | 235·5<br>258·5 | 32,000<br>37,300 | 1630s | 1660s<br>1618s | 995s<br>940w | 865w | 3320s<br>1550m | — |

* Ultraviolet absorption spectra were recorded in 95% ethanol on a Hilger Uvispek spectrophotometer and/or a Beckman Model DK2 spectrophotometer.
† Infra-red measurements were made by Mr. A. C. K. Triffett using a Perkin-Elmer Model 21 double beam spectrometer with sodium chloride prism.
‡ Infra-red in $CCl_4$ solution; all others as Nujol mull. § Inflexion.

the diacetylenic compounds were found to be more stable than those containing a conjugated olefin–acetylene. In the ultraviolet there are two distinct maxima corresponding to the two chromophores of the tetraenoic compounds and a broad band with a shoulder in the spectra of the trienynoic compounds. The contribution of the diyne feature in the dienediynoic compounds is too weak to be noticeable in the strong absorption of the other chromophore and only one band appears.

The main bands of the infra-red spectra are listed in Table 1. The C=O and C=C stretching frequencies are in sufficient agreement with the frequencies of simple *trans*-2,*trans*-4-dienoic compounds. The C=O band of such acids, normally at 1690 $cm^{-1}$, is here resolved into two. The band previously reported at 870 $cm^{-1}$ (Wailes 1959) and associated with the system *trans*-CH=CH.COX is also present here.

The C≡C stretching band, normally very weak in this type of compound, could be detected in some of our compounds only after infra-red amplification.

## IV. Insecticidal Activities

Mr. R. W. Kerr of the Division of Entomology, C.S.I.R.O., tested the six amides. Individual male houseflies were dosed on the thorax with a measured volume of a kerosene solution of each amide. Where solubility did not permit the use of kerosene alone, mixtures with acetone were applied. The results which follow (Table 2) show that isomerism at $C_{8-9}$ is not critical for the tetraen-

Table 2

Summary of insecticidal activities against Musca domestica

| | |
|---|---|
| $C_5H_{11}.CH(c.)=CH.CH(c.)=CH.CH_2CH_2.CH(t.)=CH.CH(t.)=CH.CONHCH_2CH(CH_3)_2$ | Showed good knockdown properties and a toxicity of one-third that of affinin |
| $C_5H_{11}.HC(c.)=CH.CH(t.)=CH.CH_2CH_2.CH(t.)=CH.CH(t.)=CH.CONHCH_2CH(CH_3)_2$ | Very similar to the above in having good knockdown properties; toxicity similarly is one-third that of affinin |
| $C_5H_{11}.C\equiv C.C\equiv C.CH_2CH_2.CH(t.)=CH.CH(t.)=CH.CONHCH_2CH(CH_3)_2$ .. | No activity |
| $C_5H_{11}.C\equiv C.CH(t.)=CH.CH_2CH_2.CH(t.)=CH.CH(t.)=CH.CONHCH_2CH(CH_3)_2$ .. | Showed some knockdown and weak toxicity at high concentrations |
| $C_5H_{11}.C\equiv C.CH(t.)=CH.CH_2CH_2.CON(CH_3)_2$ .. .. .. .. .. | Some knockdown properties at high concentration—toxicity weak |
| $C_5H_{11}.C\equiv C.C\equiv C.CH_2CH_2.CON(CH_3)_2$ .. .. .. .. .. | Similar to the preceding but weaker in knockdown and toxicity |

amides as both *cis*-8- and *trans*-8-isomers display the same toxicity and knock-down properties. Whether or not the *cis*-bond is necessary at $C_{10}$ has yet to be decided. The lack of activity in the *iso*butylamides of the $C_{16}$ acetylenic acids was to be expected in the light of our own previous observations and the known weak activity of anacyclin. On the other hand the activity of the dimethylamides of the $C_{12}$ acetylenic acids was unexpected and we plan in the future to prepare the corresponding olefinic amides.

## V. Experimental

Microanalyses were made by the C.S.I.R.O. and University of Melbourne Microanalytical Laboratory. Melting points were determined on a Kofler heating-stage microscope and are corrected. All distillations were carried out under nitrogen. Infra-red measurements were made by Mr. A. C. K. Triffett using a Perkin-Elmer Model 21 double-beam spectrometer with sodium chloride prism. Ultraviolet absorption spectra were recorded in 95% ethanol on a Hilger Uvispek spectrophotometer and/or a Beckman Model DK2 spectrophotometer.

(*a*) *1-Bromohept-1-yne* (*III*).—Hept-1-yne was brominated with sodium hypobromite after Straus, Kollek, and Heyn (1930), but with a 24 hr reaction time the yield was increased to 73%.

(*b*) *Dodeca-4,6-diyn-1-ol* (*V*).—Pent-4-yn-1-ol (48·1 g) in 95% ethanol (300 ml) was added rapidly to a stirred solution of cuprous chloride (1·15 g), *iso*butylamine (115 ml), and hydroxylamine hydrochloride (46 g) made up to 400 ml with water. 1-Bromohept-1-yne (100 g) in ethanol (80 ml) was then added dropwise over 1 hr keeping the temperature between 35 and 37 °C by cooling. After addition was complete the temperature was increased to 45–55 °C for 2 hr. The mixture was cooled, an aqueous solution of sodium cyanide was added, and the solution saturated with sodium chloride and extracted with ether. Distillation gave the *diynol* (62 g, 60% yield) as a colourless oil, b.p. 120–122 °C/0·2 mm, $n_D^{20}$ 1·5081 (Found: C, 80·9; H, 10·2%. Calc. for $C_{12}H_{16}O$: C, 80·9; H, 10·2%). Light absorption: $\lambda_{max.}$ 225, 237, and 252 mμ (ε 560, 450, and 250).

(*c*) *Dodeca-4,6-diynoic Acid* (*VI*).—Chromium trioxide (15 g) in concentrated sulphuric acid (24 g) and water (75 ml) was added with vigorous stirring to a solution of dodecadiynol (20·5 g) in acetone (75 ml) during 75 min, the temperature being kept at about 5 °C by ice-cooling. Stirring was continued for 45 min at room temperature, saturated sodium chloride solution was added, and the product extracted with ether and washed. The acidic fraction was extracted from the ethereal solution with 2·5% potassium hydroxide solution (6 × 50 ml) and isolated by acidification and extraction with ether as a crystalline solid (9·9 g). The ethereal solution of non-acidic material was evaporated and boiled in methanol (45 ml) with potassium hydroxide (3 g) for 90 min. The solvent was evaporated under reduced pressure and the residue taken up in water and extracted with ether. The acid, obtained from the aqueous layer as above, was a brown solid (5·7 g). Repetition of the above steps on the neutral fraction (4·5 g) gave a further 2 g of acid. Total yield was 17·6 g of crude crystalline acid. Several crystallizations from light petroleum gave *dodeca-4,6-diynoic acid* as long colourless needles (14·5 g), m.p. 66·5–68·5 °C (Found: C, 74·7; H, 8·3%. Calc. for $C_{12}H_{16}O_2$: C, 75·0; H, 8·4%).

(*d*) *N,N-Dimethyldodeca-4,6-diynamide*.—The diynoic acid from the preceding and other experiments (43·8 g) was converted to the corresponding acyl chloride by heating under reflux with oxalyl chloride (32·3 g; 10% excess) in benzene (200 ml) for 1 hr. After removal of solvent and excess oxalyl chloride by evaporation the residue was dissolved in ether (100 ml) and added with vigorous stirring to an aqueous solution of dimethylamine (150 ml; 26% w/v) cooled in ice. After ½ hr at room temperature the aqueous solution was extracted with ether and the ethereal extract washed with dilute acid, water, etc. Distillation gave the *dimethylamide* (48·1 g) as a colourless liquid, b.p. 156–162 °C/0·3 mm, $n_D^{20}$ 1·5151 (Found: C, 76·3; H, 9·4; N, 6·5%. Calc. for $C_{14}H_{21}NO$: C, 76·7; H, 9·6; N, 6·4%). This amide was tested for insecticidal activity (see Section IV).

(*e*) *Dodeca-4,6-diynal* (*VII*).—Ethyl acetate (12·1 g) in anhydrous ether (140 ml) was added over 2 hr to a solution of lithium aluminium hydride (5·2 g) in anhydrous ether (140 ml) with ice-cooling. The ethereal solution was then added over 30 min to a stirred solution of the dimethylamide above (48 g) in ether (100 ml) with ice-cooling. After 30 min at 0 °C and 30 min under reflux the mixture was hydrolysed by addition of 2N sulphuric acid at 0 °C, and the product was extracted with ether, washed with dilute acid and water, and distilled. Two main fractions were obtained: (i) 17·1 g, b.p. 112 °C/0·2–0·3 mm, $n_D^{20}$ 1·5047; (ii) 19·9 g, b.p. 162 °C/0·4 mm, $n_D^{20}$ 1·5129. Fraction (i) formed a bisulphite compound which on decomposition with alkali and distillation of the liberated oil gave pure *dodecadiynal*, $n_D^{20}$ 1·5041 (Found: C, 81·8; H, 9·3%. Calc. for $C_{12}H_{16}O$: C, 81·8; H, 9·2%). Fraction (ii) was unreduced dimethylamide.

(*f*) *Hexadeca*-trans-*2*,trans-*4-diene-8,10-diynal* (*XI*).—1-Methoxybut-1-en-3-yne (10·5 g; 30% excess) in dry tetrahydrofuran (10 ml) was added dropwise to a stirred solution of ethyl magnesium bromide (from 2·4 g magnesium and 12 g ethyl bromide) in tetrahydrofuran, the mixture being maintained at about 40 °C. After stirring at room temperature for a further 1 hr, dodeca-4,6-diynal (17·1 g) in tetrahydrofuran (10 ml) was added dropwise over 20 min with ice-cooling. The mixture was stirred at room temperature for 1½ hr, then cooled to 0 °C and ethanol (5·05 ml; 90% of theory) was added, followed after 30 min by lithium aluminium hydride (3·7 g). Stirring at room temperature was continued overnight, cooled to 0 °C, and added ethyl acetate, followed by water, aqueous tartaric acid, and ether. The aqueous layer was extracted twice more with ether, the extracts were combined, washed with water, etc. Distillation of part of the product gave the *dienediynal*, b.p. 161–162 °C/0·2 mm, $n_D^{20}$ 1·5536, and left a large (50%) non-volatile residue, but the bulk of the product (21·2 g) was oxidized without further purification.

(*g*) *Hexadeca*-trans-*2*,trans-*4-diene-8,10-diynoic Acid* (*XII*).—The crude aldehyde (21·2 g) from (*f*) above in ethanol (200 ml) was added to a solution of silver nitrate (25·5 g) in water (20 ml) and ethanol (500 ml). Sodium hydroxide (9·9 g) in water (20 ml) and ethanol (400 ml) was then added over 20 min in a stream of nitrogen. The black suspension was shaken for 20 hr with exclusion of air, after which solids were filtered off and washed and the filtrate was freed of solvent under reduced pressure. The residue was taken up in water (200 ml) and extracted with light petroleum (6 × 50 ml), acidified, and extracted with ether (2 × 50 ml). Evaporation after washing and drying ($Na_2SO_4$) gave the *dienediynoic acid* (10·5 g) which tended to polymerize during crystallization from mixtures of light petroleum with benzene, ether, or carbon tetrachloride. Fine white needles were obtained (3·0 g), m.p. 141–142 °C (Found: C, 78·5; H, 8·1%. Calc. for $C_{16}H_{20}O_2$: C, 78·7; H, 8·3%). The iso*butylamide* (from acetone) had m.p. 114·5–115 °C (Found: C, 80·0; H, 10·0; N, 5·0%. Calc. for $C_{20}H_{29}NO$: C, 80·2; H, 9·8; N, 4·7%).

(*h*) *Hexadeca*-trans-*2*,trans-*4*,cis-*8*,cis-*10-tetraenoic Acid* (*XIII*).—The dienediynoic acid (0·3 g) above in methyl acetate (10 ml) with Lindlar's catalyst (0·1 g) and quinoline (0·1 g) absorbed 59·5 ml of hydrogen at 764 mm and 20 °C (2·0 mol) before interruption. After filtering, the quinoline was removed by extraction with dilute acid and the solvent layer was washed, dried, and evaporated. Five crystallizations of the solid residue from light petroleum gave colourless plates (0·15 g) of the *tetraenoic acid*, m.p. 68·5–70·5 °C (Found: C, 77·3; H, 9·6%. Calc. for $C_{16}H_{24}O_2$: C, 77·4; H, 9·7%). The iso*butylamide*, obtained by partial reduction of the *iso*-butylamide of the dienediynoic acid and also directly from the tetraenoic acid, crystallized from acetone or acetonitrile and had m.p. 70·5–73·5 °C. It had a tendency to form fine crystals with a gel-like consistency on crystallization (Found: C, 79·1; H, 10·8; N, 4·7%. Calc. for $C_{20}H_{33}NO$: C, 79·2; H, 11·0; N, 4·6%).

(*i*) *3-Chloro-2-heptynyltetrahydropyran* (*XVIII*).—Hept-1-yne (52 g) in ether (300 ml) was added with cooling to ethyl magnesium bromide (from 13·2 g magnesium and 65 g ethyl bromide) in ether and the solution was heated under reflux until evolution of ethane had ceased (1 hr). The temperature was lowered to −10 °C and 2,3-dichlorotetrahydropyran (70 g) in ether (100 ml) was added dropwise into the centre of the liquid with vigorous stirring. After ¾ had been added a cloudiness appeared, and after complete addition the colour was milky-white but none of the polymeric material noted by Crombie and Harper (1950*a*) was present. The solution was then set aside overnight at room temperature. Saturated ammonium chloride solution was then

added slowly followed by concentrated hydrochloric acid (10 ml). The ether layer was separated, washed, and dried. Distillation gave a colourless oil (76·5 g), b.p. 86–88 °C/0·2 mm, whose various fractions showed $n_D^{20}$ 1·4885–1·4822 (mixture of *cis*- and *trans*-forms).

(*j*) *Dodeca*-trans-*4-en-6-yn-1-ol* (*XIX*).—Sodium (18·4 g; 2·2 g-atom) was powdered under xylene, rinsed several times with dry ether when cool, and covered with the same solvent. 3-Chloro-2-heptynyltetrahydropyran (76·4 g) was then added at a rate sufficient to keep the ether refluxing. After addition, the mixture was heated under reflux a further ½ hr, then cooled, and solid $CO_2$ was added followed by water. The top layer was separated, washed well, and evaporated. Distillation gave dodeca-*trans*-4-en-6-yn-1-ol (35·1 g) as a viscous oil, b.p. 102–105 °C/0·3–0·4 mm, $n_D^{20}$ 1·4898 (Riobé (1953) gives b.p. 147 °C/12 mm, $n_D^{13}$ 1·4940). The infra-red spectrum showed the expected bands at 3350 (OH), 2210 (C≡C), and 955 (*t.*=CH) cm$^{-1}$.

(*k*) *Dodeca*-trans-*4-en-6-ynoic Acid* (*XX*).—The alcohol from above (35 g) in acetone (130 ml) was oxidized at 0–5 °C under nitrogen with a mixture of chromium trioxide (25·4 g) and sulphuric acid (40·6 g) made up to 130 ml with water, exactly as described above for dodeca-4,6-diyn-1-ol. The acidic fraction was separated (11·9 g) and the neutral material was hydrolysed, the acidic fraction from the hydrolysis isolated (8·7 g) and the neutral compounds were reoxidized as above. Finally, after separating the acidic fraction from the oxidation, the neutral products were again hydrolysed and the acids isolated. In this way a total of 27 g of acidic material was obtained as a brown oil, together with 4·7 g of neutral material. Fractionation of the acids by low-temperature crystallization from light petroleum gave only 2·9 g of colourless crystalline *dodeca*-trans-*4-en-6-ynoic acid* and 18·9 g of liquid acid together with 5·2 g of petroleum-insoluble material. The non-crystallizing acids from two experiments (24 g) were esterified with methanolic sulphuric acid and chromatographed on a column of alumina (treated with 10% of 10% aqueous acetic acid), eluting with hexane, hexane+5% ether, hexane+10% ether, and finally 100% ether. With pure hexane 14·7 g of colourless oil was eluted, with hexane+5% ether and 10% ether very little was eluted, while pure ether eluted another 4·3 g of oil, partly insoluble in light petroleum. The first fraction was hydrolysed and crystallized at low temperature (—25 °C) from light petroleum and again at —5 °C to give 1·1 g of crystalline acid. The remainder was an oil, the absorption spectrum of which ($\lambda_{max.}$ 227·5 mμ, ε 16,000) was very similar to that of the crystalline acid. However the band at 960 cm$^{-1}$ (*trans*=C—H) in the infra-red spectrum of the liquid acid was very much weaker than the corresponding band in the spectrum of the crystalline acid. The liquid acid was not investigated further. Pure dodeca-*trans*-4-en-6-ynoic acid had m.p. 61–62 °C (see Table 1) (Found: C, 74·3; H, 9·2%. Calc. for $C_{12}H_{18}O_2$: C, 74·2; H, 9·3%).

(*l*) NN-*Dimethyldodeca*-trans-*4-en-6-ynamide*.—Crystalline dodec-4-en-6-ynoic acid (5·26 g) was converted into the acyl chloride with oxalyl chloride (3·8 g) in dry benzene (50 ml) under reflux and then into the dimethylamide with excess 26% aqueous dimethylamine (see (*d*) above). The product was extracted with potassium carbonate solution (giving 0·28 g of unreacted acid), washed with water, dried ($Na_2SO_4$), and distilled to give the *dimethylamide* (5·0 g) as an oil, b.p. 142–143 °C/0·3 mm, $n_D^{20}$ 1·4995. Light absorption: $\lambda_{max.}$ 228·5, 235 (inflex.) mμ (ε 18,100, 15,100). The dimethylamide was tested for insecticidal activity (see Section IV).

(*m*) *Dodeca*-trans-*4-en-6-ynal* (*XXI*).—The dimethylamide above (5·55 g from two experiments) was dissolved in dry ether (30 ml) and an ethereal solution of lithium diethoxyaluminohydride (from 16 ml of 1·13M lithium aluminium hydride solution and 1·77 ml of ethyl acetate in 20 ml ether) was added with ice-cooling over 30 min. After stirring for 40 min at 0 °C and 30 min under reflux the solution was cooled to 0 °C and 2N sulphuric acid was added. The upper layer was separated, washed with brine and with water, dried ($Na_2SO_4$), and distilled to give *dodeca*-trans-*4-en-6-ynal* (3·4 g), b.p. 94–96 °C/0·1 mm, $n_D^{20}$ 1·4880 (Found: C, 80·7; H, 10·4%. Calc. for $C_{12}H_{18}O$: C, 80·9; H, 10·2%). Light absorption: $\lambda_{max.}$ 228·5, 233 (inflex.) mμ (ε 17,000, 15,300).

(*n*) *Hexadeca*-trans-*2*,trans-*4*,trans-*8-trien-10-ynal* (*XXIV*).—Dodec-4-en-6-ynal (3·2 g) in tetrahydrofuran (5–6 ml) was added over 20 min with ice-cooling to methoxybutenynyl magnesium bromide (from 2·1 g of 1-methoxybut-1-en-3-yne and ethyl magnesium bromide as in (*f*)) in tetrahydrofuran (25 ml). The mixture was stirred at room temperature for 2 hr. cooled in ice,

and ethanol (1·34 ml) was added slowly followed after 20 min by lithium aluminium hydride (0·95 g), added over 30 min. Stirring was continued for 3 hr after which ethyl acetate (4 ml) was added at 0 °C, followed by ether and saturated aqueous tartaric acid. The upper layer was separated and combined with two more extracts of the aqueous layer, washed, dried ($Na_2SO_4$), and evaporated. The crude *trienynal* was oxidized immediately with silver oxide.

(*o*) *Hexadeca*-trans-*2*,trans-*4*,trans-*8*-*trien*-*10*-*ynoic Acid* (*XXV*).—The aldehyde from (*n*) was oxidized as in (*g*) with silver oxide (from 5·5 g of silver nitrate). The acidic fraction was a semisolid (3·0 g) from which pure *hexadeca*-trans-*2*,trans-*4*,trans-*8*-*trien*-*10*-*ynoic acid* (1·1 g) was obtained as colourless plates, m.p. 119·5–120 °C, by several crystallizations from light petroleum (b.p. <40 °C) and ether mixtures (Found: C, 77·8; H, 9·1%. Calc. for $C_{16}H_{22}O_2$: C, 78·0; H, 9·0%). The iso*butylamide* (from light petroleum–ether mixtures) had m.p. 114–116 °C (Found: C, 79·5; H, 10·3; N, 4·8%. Calc. for $C_{20}H_{31}NO$: C, 79·7; H, 10·4; N, 4·7%).

(*p*) *Hexadeca*-trans-2,trans-*4*,trans-*8*,cis-*10*-*tetraenoic Acid* (*XXVI*).—The acid from (*o*) (0·52 g) was partially hydrogenated in ethyl acetate (15 ml) in the presence of Lindlar's catalyst (70 mg) and quinoline (25 mg). The acid was over-reduced and many crystallizations from light petroleum (b.p. <40 °C) were necessary before *hexadeca*-trans-*2*,trans-*4*,trans-*8*,cis-*10*-*tetraenoic acid* (0·14 g) of satisfactory purity was obtained. The pure acid had m.p. 60·5–62·5 °C (Found: C, 77·7; H, 9·8%. Calc. for $C_{16}H_{24}O_2$: C, 77·4; H, 9·7%). The iso*butylamide*, prepared by partial reduction of the *iso*butylamide of the trienynoic acid above, and also from the tetraenoic acid, crystallized from light petroleum and had m.p. 68–69 °C (Found: C, 79·2; H, 10·9; N, 5·0%. Calc. for $C_{20}H_{33}NO$: C, 79·2; H, 11·0; N, 4·6%).

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# TERPENOID CHEMISTRY

## III. THE ESSENTIAL OILS OF EUCALYPTUS DEGLUPTA BLUME AND E. TORELLIANA F. MUELL.

By M. D. SUTHERLAND,* L. J. WEBB,† and J. W. WELLS*

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*Summary*

The essential oils of two eucalypts, which are exceptional in that they normally grow within the margin of tropical rain-forest, have been examined. The oil from *Eucalyptus deglupta* Blume was found to contain *iso*valeraldehyde, (±)-α-pinene, (—)-α-phellandrene, *p*-cymene, ocimene, (—)-carvotanacetone, and (+)-nerolidol. The oil from *E. torelliana* F. Muell. contained benzaldehyde, (±)- and (+)-α-pinene, (—)-β-pinene, ocimene, (+)-aromadendrene, and sesquiterpene alcohols. Of these constituents, ocimene, carvotanacetone, and nerolidol have not previously been identified in eucalyptus oils.

The conclusion is reached nevertheless, that because of the inadequacy of the published data on eucalyptus oil compositions, no significance can be attached as yet to the presence of these unusual constituents.

## I. INTRODUCTION

The 500 *Eucalyptus* spp. recognized by Blakely (1955) have become adapted to a great diversity of habitats on the Australian continent, but only about one-tenth extend to the tropics, and of these, few mix with wet tropical rain-forest species of Indo-Malaysian origin. On the other hand, in extratropical regions eucalypts form extensive transitions (" wet sclerophyll forests ") with temperate rain-forest elements. The genus is regarded by Herbert (1929) as having originated in the " well-watered temperate portions " of Australia, and it may be supposed that the physiological changes accompanying the unusual migration of *Eucalyptus* species to a humid tropical environment could involve modifications of the " typical " essential oil composition.

An attempt was accordingly made to use ecological criteria as a guide to the presence of novel constituents in tropical species of *Eucalyptus* occurring in exceptional habitats. Perhaps the two best examples are *E. torelliana* F. Muell. from north Queensland, and *E. deglupta* Blume from parts of New Guinea, the Celebes, and the Philippines. Both species grow on well-drained soils within the margins of wet tropical rain-forest, in which, however, they do not regenerate without disturbance of the forest cover. Because of their value as timbers these species are now grown in forestry plantations. The oils from both species were examined in as much detail as the small samples (*c.* 50 ml) available permitted. Gas chromatography, which would have enabled more comprehensive examination, was not available at the time of investigation.

* Department of Chemistry, University of Queensland, Brisbane.

† Plant and Soils Laboratory, Division of Plant Industry, C.S.I.R.O., Brisbane.

## II. Eucalyptus deglupta

The oil from *E. deglupta* gathered at Lae, New Guinea, was subjected to fractional distillation at reduced pressure, being cut into 20 fractions. The terpene fraction which amounted to 40 per cent., permitted the identification of four terpenes. α-Pinene and α-phellandrene were identified in the usual way. *p*-Cymene was isolated by removal of olefinic impurities as non-volatile acids formed by free radical addition of thioglycollic acid (Davenport, Jones, and Sutherland 1949). The presence of ocimene, indicated by ultraviolet absorption, was confirmed by thermal isomerization to *allo*ocimene which was identified as the maleic anhydride adduct. *iso*Valeraldehyde was found in the cold trap from the terpene distillation.

The presence of at least four terpene alcohols was indicated by chromatography of the azourethanes (Davenport and Sutherland 1950) prepared from a wide-boiling oxygenated terpene fraction but none could be identified. Carvotanacetone was isolated as the dinitrophenylhydrazone.

A large proportion (*c.* 50 per cent.) of the oil consisted of nerolidol, identified as a new and convenient coloured derivative, the azourethane, m.p. 62–63 °C.

The refractive index–density curve (Fig. 1) indicated the presence of at least eight components, but owing to the necessarily wide boiling point ranges of most of the fractions, this is a gross oversimplification. Thus while the percentage of sesquiterpenes was low (*c.* 5 per cent.), gas chromatography of fractions 8 and 9 showed that there were at least eight sesquiterpenes, some of which could be tentatively identified by retention times, solubility in 50 per cent. silver nitrate solution, etc. as aromadendrene, caryophyllene, and humulene.

A second sample of *E. deglupta* oil from Gadgarra, north Queensland, yielded a rather different oil of much higher optical rotation and lower ester number after acetylation. When submitted to gas chromatography, a number of the prominent constituents could be identified by retention times etc. as those of α-pinene, α-phellandrene, and caryophyllene, but such identifications should be regarded as basically unsound in the absence of crystalline derivatives. On the other hand, the absence of nerolidol in this oil could be concluded with certainty. No sample of the crude oil from Lae was available for comparison by this method, but it is obvious that the two oils were of very different composition.

## III. Eucalyptus torelliana

The essential oil was submitted to fractional distillation in an efficient column and cut into about 20 fractions. The refractive index–density diagram (Fig. 1) indicated at least 11 components, of which five have been identified.

α-Pinene, (+) and (±), comprised the bulk of the terpene fraction but the first two fractions boiling slightly below α-pinene showed an absorption maximum at 244 mμ due to the presence of benzaldehyde, identified as the dinitrophenylhydrazone.

Since benzaldehyde boils substantially (*c.* 20 °C) above α-pinene, its presence in these fractions necessitates azeotrope formation. Horsley (1952) quotes two references from the work of Lecat, the earlier (1936) listing an azeotrope at

atmospheric pressure containing 10 per cent. of benzaldehyde while the later (1949) tabulation indicates the absence of azeotropy.

Fractional distillation of a mixture of benzaldehyde and α-pinene at 50 mm pressure and moderate reflux ratio, confirmed that an azeotrope (12 per cent. benzaldehyde by weight) was formed, of boiling point approximately 1 °C below that of α-pinene at that pressure.

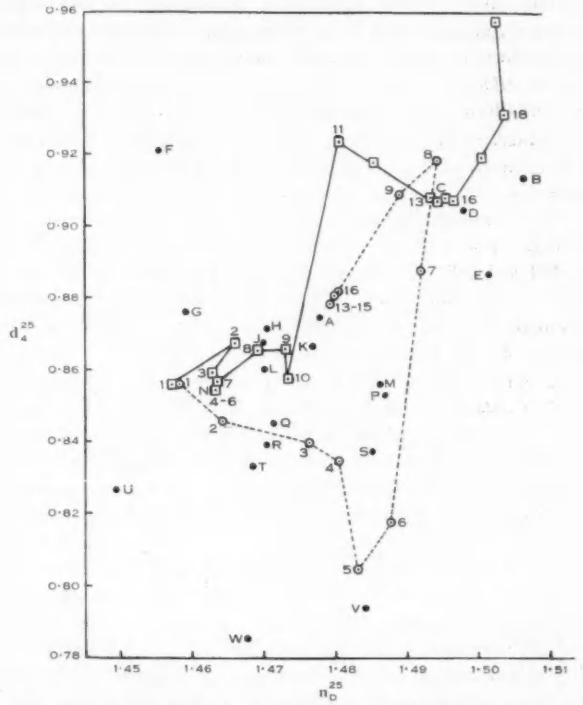


Fig. 1.—Refractive index–density plots of the distillation fractions from *Eucalyptus deglupta* and *E. torelliana*. ⊙——⊙ *E. deglupta*. ⊡—⊡ *E. torelliana*.

*A*, Nerolidol; *B*, δ-cadinene; *C*, aromadendrene; *D*, humulene; *E*, caryophyllene; *F*, 1,8-cineole; *G*, tricyclene; *H*, α-pinene–benzaldehyde azeotrope; *J*, camphene; *K*, β-pinene; *L*, 3-carene; *M*, terpinolene; *N*, α-pinene; *P*, *p*-cymene; *Q*, γ-terpinene; *R*, limonene; *S*, β-phellandrene; *T*, α-phellandrene; *U*, α-thujene; *V*, ocimene; *W*, myrcene.

The presence of a third unidentified substance in fractions 1 and 2 was ndicated by their displacement from the azeotrope–pinene fractionation line in Figure 1.

The distillation curve of fractions 6, 7, 8, and 9 (see Fig. 1) and the boiling point and optical rotation of fraction 9 suggested the presence of β-pinene which was confirmed by the isolation of nopinone as the dinitrophenylhydrazone from

the ozonolysis products of fraction 8. While fraction 8 would appear to be rich in camphene, the diversion of fraction 7 to the opposite side (to β-pinene) of the α-pinene–camphene fractionation line showed that the convexity was not due to camphene but to some other (unidentified) constituent, having $n_D^{25}$ *c.* 1·461 and $d_4^{25}$ *c.* 0·863. This was supported by the absence of camphenilone from the ozonolysis products of fraction 8.

Fraction 10 showed strong ultraviolet absorption at 235 mμ (ε 6010) consistent with the presence of 27 per cent. of ocimene. Heating at 175 °C brought about the characteristic change of absorption spectrum associated with thermal isomerization to *allo*ocimene. Although the maleic anhydride adduct was not obtained in crystalline form, the presence of ocimene was very probable.

A very interesting feature of the ultraviolet absorption spectrum of fraction 10 was the occurrence of a peak at 268 mμ and a shoulder at 279 mμ. Neither *allo*ocimene nor *p*-cymene could be responsible for this absorption. Moreover, if the absorption due to 27 per cent. ocimene is subtracted from observed curve, a curve is obtained with maxima at 250 (ε *c.* 1000) and 268 mμ (ε *c.* 1000) and shoulders at 260 and 278 mμ. Apart from the maximum at 250 mμ the curve is suggestive of a conjugated triene. A simple extension of Woodward's rules to conjugated trienes leads to the conclusion that the spectrum may be that of a disubstituted acyclic conjugated triene (calc. $\lambda_{max.}$ 266 mμ).

The wide boiling range and small quantity (1 ml) of fraction 10 would render this observation of doubtful value, had it not been observed in this laboratory on other occasions that the prolonged fractional distillation of ocimene appears to generate a close boiling impurity of similar absorption spectrum. Further investigation of this matter and of the *cis* and *trans* isomers of ocimene, is planned. Fraction 11 contained some ester and carbonyl compounds which could not be identified.

The convexity formed by fractions 12–16 (see Fig. 1) is due to the presence of aromadendrene, identified as the crystalline *apo*aromodendrone and the dinitrophenylhydrazone, m.p. 233–234 °C (decomp.). The dinitrophenylhydrazone of *apo*aromodendrone is described in the literature (Murray and Stanley 1952) as melting at 214 °C, the method of preparation being not stated. This melting point could not be confirmed. It was found that the derivative, m.p. 233–234 °C (decomp.), was obtained by Brady and Allen's methods and also by the use of dinitrophenylhydrazine in refluxing pyridine. Thus *apo*aromadendrone does not undergo ring fission when the acidic reagents are used to prepare the dinitrophenylhydrazone.

The higher boiling fractions contained at least four sesquiterpene alcohols which were separated as azourethanes but could not be identified.

## IV. Discussion

The quantitative composition of the oils from the two species is summarized in Table 1, which shows also the frequency of occurrence of the various constituents in previously examined eucalyptus oils. These percentages are largely based on the data for over 200 *Eucalyptus* spp. as summarized by Guenther (1950). α-Pinene of 67 per cent. frequency in eucalyptus oils, was present in

both oils. Cineole, which has been found in about 77 per cent. of oils examined, was absent. Ocimene, carvotanacetone, and nerolidol are recorded for the first time, and β-pinene and benzaldehyde have been recorded only once previously.

The significance of the occurrence of these constituents of apparently low frequency is far from clear. One possibility is that their presence supports the view that the atypical ecology of *E. deglupta* and *E. torelliana* is reflected in the unusual composition of the essential oils. However two additional factors must be taken into consideration. The essential oils of less than one-half of the species of *Eucalyptus* have been examined, and the greater part of the information

TABLE 1

COMPOSITION OF E. DEGLUPTA AND E. TORELLIANA OILS

| Composition | *E. deglupta* | *E. torelliana* | Frequency of Occurrence in Eucalyptus Oils* (%) |
|---|---|---|---|
| Terpenes .. .. | 40%, including | 75%, including | |
| | (±)-α-Pinene | 47% (+)- and (±)-α-Pinene | 67 |
| | | 4% (—)-β-Pinene | 0·5 |
| | (—)-α-Phellandrene | | 33 |
| | *p*-Cymene | | 10 |
| | 5% Ocimene | 1% Ocimene | 0 |
| Oxygenated terpenes | 4%, including | | |
| | 1% (—)-Carvotanacetone | | 0 |
| Sesquiterpenes .. | 5% | 18%, including | |
| | | 9% (±)-Aromadendrene | Frequent |
| Sesquiterpene alcohols | 50%, including | 4% | Frequent |
| | 45% (+)-Nerolidol | | 0 |
| Others .. .. | 1% *iso*Valeraldehyde | | 13 |
| | | 1% Benzaldehyde | 0·5 |

* Excluding *E. deglupta* and *E. torelliana*.

available, which was compiled by Baker and Smith prior to 1920, is in some ways inadequate by modern standards. This may be illustrated by reference to *E. dives* type, for which Baker and Smith (1920) recorded α-phellandrene, pinene, and cineole (considering only the terpene fraction). In Guenther's (1950) compilation, the list was extended to include camphene, cymene, dipentene, α-thujene, and γ-terpinene. A recent examination (Owen and Sutherland 1954, unpublished data) provided evidence for additional terpenes, namely, myrcene, β-phellandrene, and terpinolene.

The low frequency percentages for ocimene, carvotanacetone, and nerolidol may thus be simply a reflection of the inadequate methods of examination

necessarily employed in earlier researches, and are probably unreliable. The use of efficient fractional distillation techniques coupled with analytical and preparative gas chromatography would probably result in the detection and isolation of many additional constituents.

It is therefore not yet possible to generalize about the significance of the unusual composition of the oils of *E. torelliana* and *E. deglupta* in relation to their humid tropical origin. This reservation must also apply to previous generalizations on evolutionary status (McNair 1942) and phylogeny (Baker and Smith 1920) which are based on reported eucalyptus oil compositions.

A systematic reexamination of the eucalypts by modern techniques, with the inclusion of the less accessible species in northern Australia, would seem to be a prerequisite for the development of well-founded generalizations. Ecological criteria might then fruitfully supplement the taxonomic and genetical data in any interpretations which would then be possible. It is of economic interest to note that *E. deglupta* is a potential commercial source of the valuable perfumery material, nerolidol.

## V. Experimental

(*a*) *Isolation of the Essential Oil of* E. deglupta.—Leaves (50 lb) were collected in Memorial Avenue, Lae, New Guinea, from trees grown from seed obtained from Keravat, New Britain. The shade-dried leaves (20 lb) were steam distilled with cohobation to yield 52 ml (0·23% v/w) of pale brown oil of $d_4^{25}$ 0·8647, $n_D^{25}$ 1·4789, $[\alpha]_D$ −3·3°, acid number 2·9, ester number 17·9 and after acetylation 93·1.

Leaves (5 lb) collected from a tree at Gadgarra forestry plantation north Queensland yielded 8·5 ml (0·37% v/w) of pale yellow oil of $d_4^{25}$ 0·8609, $n_D^{25}$ 1·4725, $[\alpha]_D$ −51°, acid number 1·9, ester number 16·9 and 65 after acetylation.

(*b*) *Fractionation of* E. deglupta *Oil.*—A portion (45 ml) of the oil was fractionated through a still with 1 m of Bower-Cooke packing under reduced pressure, the significant fractions listed in Table 2 being obtained.

(*c*) *Identification of* E. deglupta *Constituents.*—(i) iso*Valeraldehyde.* The dry-ice trap condensate (3 ml) from the distillation of the lower-boiling fractions gave a positive Schiff's test and yielded a mixture of dinitrophenylhydrazones which were chromatographed on bentonite–kieselguhr. The principal zone gave yellow needles, m.p. 124–125 °C, from ethanol which did not depress the melting point of authentic *iso*valeraldehyde dinitrophenylhydrazone.

(ii) (±)-α-*Pinene.* A portion of fraction 1 (1 ml) was oxidized after Delepine (1936) to yield an acid fraction (0·50 g) which, after two recrystallizations from benzene, gave colourless needles, m.p. 104–104·5 °C, undepressed on admixture with authentic (±)-pinonic acid.

(iii) (—)-α-*Phellandrene.* Fraction 2 ($\varepsilon_{max.}$ 1090 at 263 mμ) treated in the usual way for the preparation of α-phellandrene nitrosite, yielded a crystalline solid which was recrystallized from methanol–chloroform to give 20 mg colourless needles, m.p. 107–108 °C (decomp.), when inserted at 101 °C. In chloroform solution, the nitrosite showed the rotational changes characteristic of α-phellandrene nitrosite ($[\alpha]_D^{30}$ +119 to −129° in the course of 6 hr ; *c*, 0·2).

(iv) p-*Cymene.* Fraction 4 (0·37 g) was shaken with thioglycollic acid containing a trace of benzoyl peroxide for 2 hr. The mixture was added to excess sodium hydroxide solution and steam distilled. The recovered oil (0·35 ml) was again treated to yield 0·3 ml of oil, the ultraviolet absorption ($\varepsilon_{max.}$ 415, 390, 406, and 350 at 273, 267, 264·5, and 259 mμ in ethanol) of which corresponded closely to *p*-cymene. Oxidation of a portion (21·6 mg) with 25% nitric acid at 180 °C for 8 hr afforded 6·4 mg terephthalic acid, m.p. >360 °C, converted by diazomethane to the dimethyl ester, m.p. and mixed m.p. 140–141 °C.

(v) *Ocimene.* Fraction 5 (0·72 g) was heated under nitrogen in an ampoule at 175 °C for 3·5 hr. The ultraviolet spectrum of the oil had changed from ε 18,200 at 235 mμ to ε 17,300 at 276 mμ with shoulders at 267 and 287 mμ. A portion (0·57 g) of the isomerized oil was heated in a sealed tube at 100 °C with maleic anhydride (0·17 g) for 1 hr. Vacuum sublimation yielded a colourless crystalline fraction (bath temperature 180–190 °C/2 mm) which gave colourless needles (19·7 mg), m.p. 81–82 °C, which was raised by further recrystallization to 82–83 °C, not depressed on admixture with authentic *allo*ocimene adduct.

TABLE 2

DISTILLATION FRACTIONS FROM E. DEGLUPTA OIL

| Fraction No. | Boiling Point (°C/mm) | Volume (ml) | $n_D^{25}$ | $d_4^{25}$ | $[\alpha]_D$ |
|---|---|---|---|---|---|
| 1 | 86·5–95/100 | 1·5 | 1·4580 | 0·8560 | |
| 2 | –105·5/100 | 1·5 | 1·4644 | 0·8456 | |
| 3 | –107·6/100 | 1·0 | 1·4761 | 0·8395 | |
| 4 | –109/100 | 1·0 | 1·4804 | 0·8346 | —25·6° |
| 5 | –60/10 | 2·0 | 1·4831 | 0·8040 | |
| 6 | –68·5/10 | 1·5 | 1·4868 | 0·8174 | |
| 7 | –99/10 | 2·0 | 1·4919 | 0·8876 | +6·8° |
| 8 | –119/10 | 2·0 | 1·4941 | 0·9172 | —7·2° |
| 9 | –132·5/10 | 1·5 | 1·4887 | 0·9089 | —19·8° |
| 10* | –126/5 | 0·9 | 1·4811 | | |
| 11 | –126/5 | 1·1 | 1·4800 | 0·8801 | |
| 12 | –126/5 | 1·1 | 1·4797 | | |
| 13 | –127/5 | 1·0 | 1·4793 | 0·8780 | |
| 14 | –127/5 | 1·4 | 1·4792 | | |
| 15 | –127·5/5 | 1·6 | 1·4793 | 0·8777 | +12·7° |
| 16 | –128/5 | 1·9 | 1·4796 | | |
| 17 | –128/5 | 1·7 | 1·4804 | 0·8816 | |
| 18 | –126/2 | 0·5 | 1·4837 | | |
| 19 | –126/2 | 0·2 | 1·4859 | | |
| 20 | –135/2 | 0·5 | 1·4918 | | |

* Distillation from fraction 10 onwards was conducted in a semimicrostill (20 cm of Bower-Cooke packing), using only a portion (12 ml) of the residual oil (21 ml).

(vi) (—)-*Carvotanacetone.* Fraction 7 (0·5 g) was treated with an excess of Brady's reagent to yield red crystals (55 mg) of m.p. 160–170 °C. Three recrystallizations from methanol–ethyl acetate raised the m.p. to 191–192 °C, which was not depressed by admixture with the dinitrophenylhydrazone of (—)-carvotanacetone. The infra-red spectra (Nujol mulls) of the two derivatives were also consistent with their identity (Found: C, 58·3; H, 6·1; N, 16·7%. Calc. for $C_{16}H_{20}N_4O_4$: C, 57·8; H, 6·1; N, 16·9%).

(vii) *Terpene Alcohols.* Treatment of fraction 8 with phenylazophenyl *iso*cyanate in pyridine yielded a mixture of azourethanes which were separated by chromatography in light petroleum–benzene on alumina into four zones, none of which could be identified with known substances.

(viii) (+)-*Nerolidol.* A portion of fraction 14 was oxidized by a slight modification of the procedure of Stoll and Commarmont (1949) to crude farnesal, the dinitrophenylhydrazone of which, after five recrystallizations, showed m.p. 102–103 °C with sintering at 99 °C. A mixture with the authentic farnesal derivative (m.p. 103–104·5 °C) melted at an intermediate temperature.

The phenylazophenyl urethane, prepared as in (*d*), formed yellow rosettes, m.p. 62–63·5 °C, not depressed on admixture with authentic urethane.

(*d*) *Preparation of* (+)-*Nerolidol Azourethane.*—Two grams of nerolidol ($[\alpha]_D^{20}$ +14·7°, from *Melaleuca viridiflora* oil (Jones and Haenke 1936)) was mixed with a 50% excess of phenylazophenylurethane and two drops of pyridine. After standing at 5 °C for 2 days, water was added and a day later the reaction mixture was worked up by repeated extraction with light petroleum (b.p. <40 °C). The combined extracts were washed with citric acid solution until the aqueous layer remained colourless. The dried solution was allowed to evaporate slowly at room temperature and deposited light yellow crystals (2·8 g), m.p. 57–61 °C. On crystallization it yielded yellow rosettes, m.p. 62–63 °C, $[\alpha]_D^{22}$ +22·7° (*c*, 1·76 in ethanol) not changed by further recrystallization (Found: C, 75·3; H, 7·8; N, 9·4%. Calc. for $C_{28}H_{35}O_2N_3$: C, 75·4; H, 7·9; N, 9·4%).

(*e*) *Isolation of the Essential Oil of* E. torelliana.—Leaves (50 lb) were collected from a tree in the Gadgarra forestry plantation. The air-dried leaves (25 lb) were steam distilled with cohobation, for 7 hr. to yield 55 ml (0·24% v/w) of pale yellow oil of $d_4^{25}$ 0·8733, $n_D^{25}$ 1·4735, $[\alpha]_D$ +9·5°, acid number 1·5, ester number 10·1 and after acetylation, 22·8.

TABLE 3

DISTILLATION FRACTIONS FROM E. TORELLIANA OIL

| Fraction No. | Boiling Point (°C/mm) | Volume (ml) | $n_D^{25}$ | $d_4^{25}$ | $[\alpha]_D$ |
|---|---|---|---|---|---|
| 1 | 76–87/100 | 0·6 | 1·4571 | 0·8556 | — |
| 2 | 72–72·5/50 | 1·5 | 1·4658 | 0·8666 | +18·5° |
| 3 | –72·5/50 | 2·2 | 1·4628 | 0·8590 | +19·7° |
| 4 | –72·5/50 | 2·0 | 1·4635 | 0·8545 | +20·2° |
| 5 | –72·5/50 | 3·5 | 1·4636 | 0·8545 | 20·1° |
| 6 | –72·5/50 | 7·0 | 1·4637 | 0·8545 | +19·9° |
| 7 | 53·4–56·9/20 | 4·5 | 1·4632 | 0·8563 | +18·9° |
| 8 | –59·3/20 | 1·0 | 1·4688 | 0·8649 | —0·6° |
| 9 | –73·4/20 | 2·0 | 1·4728 | 0·8655 | —19·9° |
| 10 | 59·4–82·5/10 | 1·0 | 1·4732 | 0·8569 | —17·3° |
| 11 | –109·5/10 | 1·0 | 1·4804 | 0·9232 | —18·6° |
| 12 | –116·7/10 | 1·0 | 1·4851 | 0·9178 | —12·9° |
| 13 | –118·2/10 | 1·0 | 1·4933 | 0·9079 | —5·8° |
| 14 | –120·2/10 | 1·0 | 1·4943 | 0·9071 | +3·2° |
| 15 | –121·5/10 | 1·0 | 1·4952 | 0·9079 | +14·4° |
| 16 | –122·8/10 | 1·0 | 1·4963 | 0·9092 | +17·1° |
| 17 | –129·8/10 | 1·0 | 1·5002 | 0·9186 | +6·2° |
| 18 | –136·4/10 | 1·0 | 1·5037 | 0·9310 | —0·0° |
| 19 | –143·3/10 | 1·0 | 1·5024 | 0·9567 | —4·7° |
| 20 | –144·5/10 | 0·5 | 1·5018 | — | — |

(*f*) *Fractionation of* E. torelliana *Oil.*—A portion of the oil (48 ml) was fractionated through a still with 1 m of Bower-Cooke packing under reduced pressure at reflux ratio *c.* 18, the significant fractions being listed in Table 3.

(*g*) *Identification of* E. torelliana *Constituents.*—(i) *Benzaldehyde.* Fraction 1 yielded a mixture of dinitrophenylhydrazones (0·10 g) which were chromatographed on bentonite–kieselguhr. The principal zone was benzaldehyde dinitrophenylhydrazone obtained as red needles from ethanol, m.p. and mixed m.p. 237–238 °C, and showing satisfactory analytical values. The ultraviolet spectrum of fraction 2 ($E_{1\,cm}^{1\%}$ 70·2 at 244 mμ in ethanol) indicated a benzaldehyde content of 4·6%, based on $\varepsilon_{max.}$ 16,250 (Morton, Hassan, and Calloway 1934; cf. Grammaticakis 1953).

(ii) (±)- *and* (+)-α-*Pinene.* Fraction 6 (1 g) was oxidized as in (*c*) (ii) to yield 0·94 g of acids, separated by crystallization into (±)-pinonic acid (0·44 g), m.p. and mixed m.p. 104–105 °C.

and 0·10 g impure (+)-pinonic acid, m.p. 57–58 °C and mixed m.p. 64–67 °C, with authentic (+)-pinonic acid (m.p. 67·5–68·5 °C).

(iii) (—)-β-*Pinene.* Fraction 8 (0·66 g) was ozonized in acetic acid to give 0·1 ml of steam volatile oil which was treated with Brady's reagent. The red precipitate (0·055 g), m.p. 140–142 °C, was chromatographed on alumina to give a broad orange zone, the front and rear portions of which were each recrystallized twice from methanol to yield nopinone dinitrophenylhydrazone, m.p. and mixed m.p. 145–146 °C.

A trial showed that nopinone and camphenilone dinitrophenylhydrazones were not separated well on this alumina but the sharp m.p.'s obtained from the fraction 8 derivative indicated the absence of the camphenilone derivative (for which we find m.p. 159–160 °C, in agreement with Archer and Hickinbottom 1954; cf. Clement 1951).

(iv) *Ocimene.* The ultraviolet spectrum of fraction 10 showed maxima at 235 mμ (ε 6010), 268 mμ (ε 1045), and 278 mμ (ε 856) indicating the presence of 27% ocimene (based on ε 22,600 at 234 mμ in ethanol for pure ocimene (Owen and Sutherland 1954, unpublished data)).

Isomerization of fraction 10 (0·5 ml) as described in (c) (v) yielded an oil with an absorption maximum of ε 6660 at 277 mμ and shoulders at 268 and 288 mμ.

TABLE 4

DISTILLATION FRACTIONS FROM PINENE–BENZALDEHYDE MIXTURE

| Fraction No. | Boiling Point (°C/50 mm) | Volume (ml) | $d_4^{25}$ | $n_D^{25}$ |
|---|---|---|---|---|
| 1 | 71·5–72·2 | 5 | 0·8711 | 1·4704 |
| 2 | –72·4 | 5·5 | 0·8712 | 1·4705 |
| 3 | –72·5 | 5 | 0·8711 | 1·4706 |
| 4 | –73·2 | 3 | 0·8699 | 1·4703 |
| 5 | –73·4 | 3 | 0·8652 | 1·4685 |
| 6 | –73·5 | 4 | 0·8552 | 1·4647 |

(v) (+)-*Aromadendrene.* Fraction 14 (0·92 g) was ozonized in acetic acid to yield 0·68 g of neutral products which were treated with Brady's reagent at 5 °C for 24 hr. The crude dinitrophenylhydrazone was chromatographed on bentonite–kieselguhr, separating into six zones, two of which crystallized. The first zone gave fine orange needles, m.p. 226–228 °C (decomp.), which after three crystallizations from ethyl acetate melted at 233–234 °C (decomp.) alone and mixed with authentic *apo*aromadendrone dinitrophenylhydrazone (see below). The second zone yielded red needles, m.p. 221–222 °C.

Fraction 15 (0·59 g) ozonized similarly yielded a neutral oil (0·45 g) which at 5 °C deposited colourless needles of *apo*aromadendrone, m.p. and mixed m.p. 81–82 °C, after recrystallization from methanol.

(vi) *Miscellaneous.* Fraction 11 (ester number 8·4) showed a positive Schiff's test and yielded two oily dinitrophenylhydrazones separable on bentonite–kieselguhr.

The presence of five high-boiling alcohols was indicated by the separation of five zones during chromatography of the azourethanes prepared from fraction 20. One zone only crystallized from light petroleum as orange crystals, m.p. 149·5–151 °C, but the analytical values were unsatisfactory (Found: C, 73·4, 72·8; H, 8·7, 8·0%).

(*h*) apo*Aromadendrone 2,4-Dinitrophenylhydrazone.* — *apo*Aromadendrone (0·5 g), m.p. 81·5–82 °C, on standing for 24 hr at 5 °C with Brady's reagent, yielded 0·85 g of a red precipitate, m.p. 212–214 °C. Three crystallizations from ethyl acetate yielded orange needles, m.p. 232–233 °C (decomp.), $[\alpha]_D^{28}$ +366° (*c*, 0·88 in chloroform) (Found: C, 62·1; H, 6·9; N, 14·5%. Calc. for $C_{29}H_{26}N_4O_4$: C, 62·1; H, 6·8; N, 14·4%).

(*i*) *α-Pinene–Benzaldehyde Azeotrope.*—A mixture of 24·9 g α-pinene ($d_4^{24}$ 0·8548, $n_D^{25}$ 1·4640) and 2·55 g freshly distilled benzaldehyde ($d_4^{25}$ 1·0432, $n_D^{25}$ 1·5438) was fractionated through a 1 m Bower-Cooke type still at 50 mm pressure using a reflux ratio of 16, to yield fractions presented in Table 4.

The coincidence of the physical constants of fractions 1, 2, and 3 indicates that these fractions possess the azeotropic composition for 50 mm pressure, this composition (12% w/w of benzaldehyde) being determined by comparison of the refractive index and density with those of mixtures prepared by weighing.

## VI. Acknowledgments

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# TERPENOID CHEMISTRY

## IV. THE TURPENTINE OF ARAUCARIA CUNNINGHAMII AIT.

By M. J. GALLAGHER* and M. D. SUTHERLAND*

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*Summary*

Contrary to Baker and Smith's (1910) statement, the turpentine from *Araucaria cunninghamii* Ait. does not contain methanes or menthenes. Identified constituents include *n*-nonane, *n*-undecane, (—)- and (±)-α-pinene, (—)-β-pinene, myrcene, (±)-limonene, terpinolene, caryophyllene, and humulene.

### I. INTRODUCTION

Baker and Smith (1910) reported an examination of the latex from the stumps of felled hoop pine, *Araucaria cunninghamii* Ait., and concluded that the volatile oil from the latex contained substances " of the $C_{10}H_{20}$ series and probably also the $C_{10}H_{18}$ series ". They were unable to identify any constituents. From a reconsideration of the physical properties and the slight chemical information reported by Baker and Smith, we considered that a $C_9$ *cyclo*olefin and a $C_9$ *cyclo*paraffin provided the best fit to the experimental data and we were interested to reexamine the volatile oil for the substances in question.

### II. RESULTS AND DISCUSSION

From several batches of hoop pine† latex the yield (0·5–1·5 per cent. v/w) of turpentine was very variable, as was also its refractive index ($n_D^{25}$ 1·434–1·463). However, gas chromatography showed that the various samples were qualitatively very similar.

One large sample was fractionally distilled in a 20 plate still, the physical properties of the fractions being measured and plotted as $n$—$d$ and $\alpha$—$d$ curves in the usual way.

About one-half of the charge distilled at a temperature (89–91 °C/100 mm) slightly below the boiling point of α-pinene, the $n$—$d$ points lying on the fractionation line between *n*-nonane and α-pinene. The presence of the former was confirmed by washing out the α-pinene with fuming sulphuric acid to yield a paraffin of correct physical properties and infra-red spectrum. In a similar way, *n*-undecane was identified in a higher boiling fraction (34) which also showed a low density and refractive index.

The physical properties of fraction 25 corresponded closely to those of α-pinene and the identity was confirmed by oxidation of the fraction to (±)-pinonic acid. Since in this case the absence of α-thujene could not be

* Chemistry Department, University of Queensland, Brisbane.

† From the stumps of plantation thinnings at Imbil, Queensland, provided by Mr. H. S. Curtis, Queensland Department of Forestry.

deduced from the $n-d$ plot of the distillation fractions, it was tested for by the method of Davenport, Jones, and Sutherland (1949) and shown to be absent.

The use of gas chromatography (Scott microflame detector with " Apiezon M " column at 170 °C) made it possible to recognize the other terpene constituents of the fractions above 25, by comparing the retention times of observed peaks with those of authentic samples.

Thus a peak corresponding to myrcene reached a maximum concentration of 40 per cent. in fraction 29, in agreement with the observed ultraviolet absorption peak at 224 mμ. The presence of myrcene was confirmed by the preparation of the maleic anhydride adduct. The myrcene peak was followed by the β-pinene peak (an inversion of the boiling point order), this constituent reaching a maximum concentration in fraction 28 and being identified by conversion to the known nopinone dinitrophenylhydrazone. A limonene peak reached maximum concentration in fraction 31, which on bromination yielded the characteristic (±)-limonene tetrabromide.

A peak of retention time characteristic of terpinolene reached a maximum concentration of 20 per cent. in fraction 32. Microsamples of this peak, isolated by gas chromatography, readily yielded the characteristic unstable terpinolene tetrabromide, convertible to the stable tetrabromide of the same melting point by remelting (Sutherland and Wells 1956).

Fraction 37 consisted largely of caryophyllene as indicated by the physical constants and the retention time of the major peak and confirmed by the preparation of the characteristic nitrolbenzylamine. The presence of humulene also, was indicated by a minor peak of appropriate retention time. By extraction with small volumes of 50 per cent. aqueous silver nitrate (Hildebrand, Sutherland, and Waters 1959) and steam distillation of the extracts, a concentrate of substantially pure humulene was obtained and was converted to the characteristic humulene trioxide.

The several samples of turpentine were submitted to gas chromatography, a typical curve being reproduced with peak identifications in Figure 1. The proportions of constituents varied between samples but this may be partly due to the variable dryness of the latex samples as received and the different periods of steam distillation employed. Myrcene appeared to be absent from some samples. Most samples showed the presence of two unidentified sesquiterpenes (?) eluting prior to caryophyllene, these being in negligible proportion in the sample examined in detail. Up to three minor constituents which eluted after humulene were noted also in some samples.

Quantitative analyses of the turpentine samples by gas chromatography were very approximate, since the terpenes were not completely resolved under the conditions employed but the following two sets of percentages (by wt.) are representative: *n*-nonane 32,14; *n*-undecane 11,11; α-pinene 32,38; β-pinene 4,9; myrcene —,4; limonene 2,3; terpinolene 3,2; caryophyllene 12,7; humulene 1,1; unknown substances A 1·5,2; B 1,6; C —,0·5; D 0·7,1·5; E —,1·5. *n*-Heptane and *n*-tridecane were absent.

Mirov (1948) and his collaborators have shown that *n*-undecane is of fairly wide occurrence in American turpentines, but *n*-nonane has not previously been

identified as a turpentine constituent though Haagen-Smit, Freeman, and Mirov (1947) reported the presence of traces of an unidentified nonane in the turpentine of *Pinus torreyana*. *n*-Nonane has been identified in the essential oils of *Sarothra gentianoides* L. (Bogert and Marion 1933) and *Pittosporum eugenoides* (Carter and Heazlewood 1949).

Analysis of the turpentine to the extent indicated above thus reveals no substance of the $C_{10}H_{20}$ or $C_{10}H_{18}$ series since all the lower boiling substances revealed by peaks in the gas chromatograms have been identified. It is now apparent that the so-called menthane of Baker and Smith was an impure *n*-nonane, enriched by the removal of most of the terpenes by autoxidation and distillation.

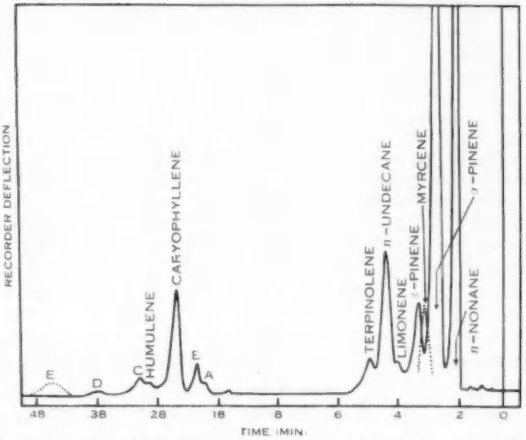


Fig. 1.—Gas chromatogram of a sample of hoop pine turpentine on " Apiezon M " at 170 °C. At 8 min, the chart speed was reduced and the sensitivity was increased by a factor of five in both cases.

## III. Experimental

(*a*) *Isolation of Turpentine.*—(i) Latex (6·6 kg ; received 8.iv.58) was boiled in excess water under an oil-trap and condenser for 9 hr to yield 97 ml of clear yellowish oil, $n_D^{22\cdot5}$ 1·4513.

(ii) Latex (0·56 kg ; received 9.iii.56) was distilled to yield 3·0 ml of oil, $n_D^{20}$ 1·4378.

(iii) Latex (3·5 kg ; received 21.viii.58) in 8 hr of distillation yielded 12·6 ml of pale yellow oil, $n_D^{21}$ 1·4633. A further 8 hr distillation yielded 3·4 ml of darker oil.

(*b*) *Fractional Distillation of Turpentine.*—A sample of oil (230 ml) was fractionally distilled through a still with 20 in. of Lecky-Ewell type packing, initially under a pressure of 100 mm and later at 10 mm. The reflux ratio was *c.* 25 with occasional periods of higher reflux ratio or total reflux. The properties of selected fractions are listed in Table 1.

(*c*) *Gas Chromatography.*—Scott's (1956) microflame detector was used with column and detector maintained at 170 °C by means of refluxing butyl cellosolve. The stationary phase consisted of 20% by weight of " Apiezon M " on " Embacel " in a 4 ft by 5 mm column of copper tubing. Nitrogen was used as a carrier gas at a flow rate of 42·5 ml per min (S.T.P.). It was mixed with hydrogen (17 ml per min S.T.P.) after leaving the column and the mixture was burnt

above a glass jet. A Sunvic recorder of 2·5 mV full-scale deflection was used. Convenient sample sizes for analytical work were of the order of 0·5–2 μl. For preparative work, the column could be heavily overloaded (up to 60 μl) without excessive loss of resolution although such large samples caused very considerable elongation of the flame with resultant distortion of the peaks. However, it was possible to check the retention times of particular peaks prior to carrying out a no-flame run in which condensation of the desired fractions from the gas stream was achieved by attaching short lengths of dry-ice-cooled, glass tubing to the jet.

(*d*) *Identification of Constituents.*—(i) n-*Nonane.* (1) Fraction 4 (5 ml) was heated on the water-bath with conc. sulphuric acid (100 ml) with mechanical stirring for 1½ hr. The undissolved oil was separated, washed with aqueous alkali, with water, and dried to yield 0·8 ml of oil of $n_D^{25}$ 1·4227 and $d_4^{25}$ 0·7539. Repeating the treatment effected no further purification.

TABLE 1

DISTILLATION FRACTIONS FROM HOOP PINE TURPENTINE

| No. | Boiling Point (°C/mm) | Volume (ml) | Cumulative Volume (ml) | $n_D^{25}$ | $d_4^{25}$ | $[\alpha]_D^{28}$ |
|---|---|---|---|---|---|---|
| 1 | 80·4–88·7/100 | 4 | 4 | 1·4402 | 0·8030 | —9·2° |
| 2 | 88·7–88·8 | 6 | 10 | 1·4409 | 0·8030 | —9·6° |
| 13 | 89 | 5 | 69 | 1·4493 | 0·8239 | —12·2° |
| 19 | 90 | 6 | 100 | 1·4585 | 0·8418 | —14·1° |
| 22 | 90·3–90·8 | 5 | 115 | 1·4615 | 0·8474 | —14·0 |
| 25 | 91·5–92·5 | 5 | 130 | 1·4645 | 0·8519 | —12·4° |
| 28 | 92–100 | 5 | 146 | 1·4708 | 0·8500 | —7·3° |
| 31 | 55–63/10 | 5 | 161 | 1·4739 | 0·8327 | —3·6° |
| 34 | 73–93 | 5 | 178 | 1·4246 | 0·7736 | —1·4° |
| 37 | 118–119 | 6 | 194 | 1·4962 | 0·8958 | —6·7° |
| 38 | 119–120 | 5 | 199 | 1·4968 | — | — |
| 39 | 120–125 | 2 | 201 | — | — | — |
| Residue: 8·5 ml of dark oil; trap: 2 ml of oil | | | | | | |

(2) Fractions 1, 5, and 7 were shaken at room temperature with fuming sulphuric acid for 1 hr. The recovered oil (2·4 ml) showed $n_D^{25}$ 1·4040 and $d_4^{25}$ 0·7153 in good agreement with the values ($d_4^{20}$ 0·7174, $n_D^{20}$ 1·4054) found by Vogel (1946). The infra-red spectrum was almost identical with that in the literature (American Petroleum Institute Project No. 44 Collection, Spectrum No. 389).

(ii) (±)-α-*Pinene.* Fraction 25 (2·03 g) was oxidized with 4·74 g of potassium permanganate by the method of Delepine (1936) to yield 1·35 g of crude crystalline acid. Recrystallization from benzene yielded pure (±)-pinonic acid, m.p. and mixed m.p. 103·5–105 °C.

(iii) (—)-β-*Pinene.* Fraction 28 was ozonized in aqueous acetic acid and the ozonide was reduced with zinc dust. The neutral products (0·99 g) were converted to dinitrophenylhydrazones by Allen's reagent, and chromatographed in benzene on bentonite–kieselguhr to yield three bands, the first of which was nopinone dinitrophenylhydrazone, m.p. 143·5–144·5 °C, and mixed m.p. 144·5–145·5 °C, with an authentic specimen (m.p. 145–146 °C) from (—)-β-pinene.

(iv) *Myrcene.* Of fraction 29 ($\varepsilon_{max.}$ 7480 at 224 mμ), 0·70 ml was refluxed in benzene with maleic anhydride (0·23 g). The resulting anhydride was converted to the dibasic acid, m.p. 118–120 °C, from acetonitrile, not depressed by an authentic specimen.

(v) (±)-*Limonene.* Fraction 31 (1·0 ml) was brominated in amyl alcohol and ether to yield dipentene tetrabromide, m.p. 124·5–125·5 °C, from ethyl acetate–methanol, not depressed by an authentic sample.

(vi) *Terpinolene.* A 20 μl sample of fraction 32 was injected under "no-flame" conditions and during the appropriate period, the jet was connected to a dry-ice-cooled glass tube drawn out at the other end to a 2–3 mm diameter capillary. Sealing the capillary tip and centrifuging down gave 3 mg (*c.* 90% recovery) of terpinolene, shown to be about 90% pure by re-injection under analytical conditions. Crystals of unstable terpinolene tetrabromide, m.p. 115–116 °C from chloroform methanol, were readily obtained from such preparations. A mixture with authentic stable terpinoline tetrabromide showed a melting point depression of about 15 °C but after cooling and remelting several times, the melting point rose to 115–116 °C, identical with that of authentic stable terpinolene tetrabromide.

(vii) n-*Undecane.* Fraction 34 partially solidified at −30 °C. The separated solid was washed repeatedly with conc. sulphuric acid and then fractionated in a semimicrostill. The distillate, b.p. 72 °C/10 mm, $d_4^{25}$ 0·7372, $n_D^{25}$ 1·4134 analysed satisfactorily for *n*-undecane and showed an infra-red absorption spectrum virtually identical with that recorded for *n*-undecane (American Petroleum Institute Project No. 44 Collection, Spectrum No. 391). For *n*-undecane, Vogel (1946) reports $n_D^{20}$ 1·4172 and $d_4^{20}$ 0·7398.

(viii) *Caryophyllene.* A portion of fraction 37 yielded from the preparation of the nitrosochloride, a blue-green oil which was treated with benzylamine to yield fine colourless needles of caryophyllene nitrolbenzylamine, m.p. 169–170 °C, $[\alpha]_D^{20}$ +206° (*c*, 1·1 in $CHCl_3$), showing no depression of melting point when mixed with an authentic sample.

(ix) *Humulene.* Extraction of fractions 36, 37, and 38 with saturated aqueous silver nitrate solution, followed by steam distillation of the aqueous layer, yielded an oil (1·5 ml) consisting of 80% caryophyllene and 20% humulene. Brief equilibration of this with 50% (w/w) aqueous silver nitrate (1·2 ml) yielded humulene (100 mg) of 97% purity, as indicated by gas chromatography. The infra-red spectrum showed all the peaks of authentic humulene. This sample was oxidized with monoperphthalic acid and yielded, after recrystallization from hexane–ethanol, humulene trioxide, m.p. and mixed m.p. 121–122 °C.

## IV. Acknowledgments

The authors wish to thank Mr. H. S. Curtis of the Queensland Department of Forestry at Imbil for his generous cooperation in providing numerous samples of hoop pine latex, and also C.S.I.R.O. for a Junior Studentship awarded to one of us (M.J.G.).

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# THE STEREOCHEMISTRY OF AROMADENDRENE, GLOBULOL, AND LEDOL

By B. A. Graham,* P. R. Jefferies,* G. J. H. Melrose,* K. J. L. Thieberg,* and D. E. White*

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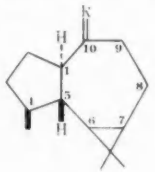
*Summary*

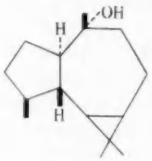
The conversion of aromadendrene, obtained from a variety of essential oils or by dehydration of globulol, to *apo*aromadendrone has been studied, together with the further degradation of *apo*aromadendrone. The reaction of *apo*aromadendrone with methyl magnesium iodide has afforded globulol and *epi*globulol. From the reactions of these compounds, structures are proposed for aromadendrene (I), *apo*aromadendrone (II), globulol (III), *epi*globulol (IV), and ledol (V).

## I. Introduction

The *cyclo*propane ring in aromadendrene, globulol, and related compounds has recently been shown to be attached to C(6) and C(7) (Büchi *et al.* 1959; Dolejš and Šorm 1959*a*). Hence the structures, which we proposed in preliminary papers (Blumann *et al.* 1954; Jefferies, Melrose, and White 1959), must be



(I: R = $CH_2$)
(II: R = O)



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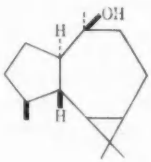

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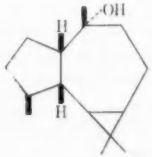

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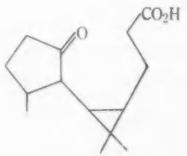


(VII)

modified to those shown for aromadendrene (I), *apo*aromadendrone (II), globulol (III), *epi*globulol (IV), and ledol (V). The dehydration of 1-methyl*apo*aromadendrol (Jefferies, Melrose, and White 1959) appears to have been accompanied by rearrangement. This reaction is now being examined further.

* Chemistry Department, University of Western Australia, Nedlands, W.A.

Meanwhile, we report the results leading to our conclusions about the stereochemistry at C(1) and C(10) in the globulol–ledol group. These are now supported by the work of Büchi *et al.* (1959) and by the late results of the Prague group (Dolejš and Šorm 1959*b*).

## II. Discussion

Globulol was first related to aromadendrene by the observation that pyrolysis of its 3,5-dinitrobenzoate afforded aromadendrene, which was identified spectroscopically and by ozonolysis to *apo*aromadendrone. Subsequently, dehydration with phosphoryl chloride in pyridine was found to be a more convenient method for the preparation of aromadendrene in quantity. The tertiary character of the hydroxyl group in globulol is confirmed by the facility of these dehydrations and by its absorption at 3615 $cm^{-1}$.

By ozonolysis of aromadendrene, Birch and Lahey (1953) obtained *α-apo*aromadendrone which was isomerized by heat or alkali to *apo*aromadendrone. In order to clarify the formation of *α-apo*aromadendrone which has not been obtained by previous workers (Briggs and Short 1928 ; Radcliffe and Short 1938 ; Naves and Perrottet 1940 ; Treibs and Barchet 1950), aromadendrene (vinylidene absorption at 3080, 1634, and 887 $cm^{-1}$) obtained by the pyrolysis of globulyl 3,5-dinitrobenzoate which was considered more likely to be free from impurities than the hydrocarbon isolated by distillation of an essential oil, was treated with osmium tetroxide. The resulting glycol on oxidation with periodic acid afforded *apo*aromadendrone (carbonyl absorption at 1699 $cm^{-1}$ ; >six-membered ring ketone) directly. Hence rearrangement during the formation of *apo*aromadendrone seemed unlikely. The direct formation of *apo*aromadendrone on ozonolysis of aromadendrene was also observed on material prepared from globulol, and on the sesquiterpene hydrocarbon fractions from the essential oils of *Eucalyptus globulus* Labill., *E. caesia* Benth., *E. nova anglica* Deane & Maiden, and *Thryptomene kochii* Pritzel. Hence we considered that *α-apo*aromadendrone was not derived from aromadendrene but from an isomeric hydrocarbon. This conclusion has now been established by Dolejš *et al.* (1959).

The structure assigned to the keto acid (VII) (Birch and Lahey 1953) was confirmed by its stepwise preparation from *apo*aromadendrone. Reduction of the ketone with lithium aluminium hydride afforded *apo*aromadendrol, which was dehydrated with phosphoryl chloride in pyridine to the hydrocarbon (VI) (trisubstituted double bond absorption at 3070, 1670, 826, 809, and 801 $cm^{-1}$). The glycol obtained by oxidation of this hydrocarbon with osmium tetroxide was cleaved with periodic acid and the product oxidized with chromic acid to the keto acid (VII). It was characterized by its absorption at 1742 $cm^{-1}$ (*cyclo*pentanone) and 1710 $cm^{-1}$ (carboxyl) and gave the same oximino acid that Birch and Lahey (1953) had obtained by the action of amyl nitrite and sodium ethoxide on *apo*aromadendrone. These degradations confirm the partial formula VIII for aromadendrene.

Information on the stereochemistry of aromadendrene and globulol was obtained from the reaction of *apo*aromadendrone with methyl magnesium iodide. The major product was *epi*globulol, which was isolated as its 3,5-dinitrobenzoate,

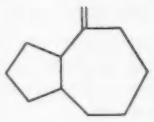
and some globulol was obtained. Brief heating of *epi*globulyl 3,5-dinitrobenzoate at 30 °C above its melting point afforded aromadendrene. This isolation of globulol and regeneration of aromadendrene from its isomer demonstrate that there was no epimerization at C(1) in aromadendrene during its conversion to *apo*aromadendrone. Thus aromadendrene, *apo*aromadendrone, globulol, and *epi*globulol must have the same configuration at the ring junction. Also, this must be the more stable configuration since Birch and Lahey (1953) found that *apo*aromadendrone was not isomerized by base.

The hydroxyl absorptions of globulol (3615 $cm^{-1}$) and *epi*globulol (3620 $cm^{-1}$) indicate that the former has a *pseudo*-equatorial hydroxyl and the latter a *pseudo*-axial hydroxyl group (Cole *et al.* 1959). This is supported by the spectra of their 3,5-dinitrobenzoates which show methyl bending absorptions at 1384 and 1377 $cm^{-1}$ respectively, indicating that the methyl group at C(10) is *pseudo*-axial in globulol and *pseudo*-equatorial in *epi*globulol (Cole and Müller 1960, personal communication). Furthermore, the product of the Grignard reaction between *apo*aromadendrone and methyl magnesium iodide, when chromatographed on acid-washed alumina, was largely dehydrated and the only alcoholic product obtained was globulol. This selective dehydration of *epi*globulol, together with its facile dehydration with sulphuric acid in acetone, indicates that it has a *trans*- and *anti*-parallel disposition of the hydrogen eliminated and the hydroxyl group on C(10). That the hydrogen eliminated is on C(1) is indicated by the oxidation of the hydrocarbon product with permanganate or with osmium tetroxide to ledglycol (IX). Hence in globulol the hydrogen on C(1) and the hydroxyl group on C(10) are *cis*.

Although it had been generally accepted that the fusion of *cyclo*pentane with *cyclo*heptane rings was more stable in the *cis*-forms (cf. Birch and Lahey 1953) it was demonstrated by Ayres and Raphael (1958) that the *trans*-form of *bicyclo*-[5,3,0]-decan-9-one (X) was the more stable. Although this compound with its carbonyl group in the five-membered ring is not a perfect model for *apo*aromadendrone, it seems to be the best available and indicates that the ring junction in *apo*aromadendrone and related compounds is more likely to be *trans*. This conclusion is supported by a study of molecular models, and is now confirmed by Büchi *et al.* (1959) and by Dolejš and Šorm (1959*b*). With the position of the *cyclo*propane ring established by Büchi *et al.* (1959) and by Dolejš and Šorm (1959*a*) and the observation of Birch and Lahey (1953) that the methyl group at C(4) must be *trans* to the group at C(5), the structures of globulol (III) and *epi*globulol (IV) are established.

The epimeric relationship of globulol and *epi*globulol and their dehydrations to the same hydrocarbon (XI) as ledol (cf. Dolejš, Šorm, and Soucek 1959) indicate that ledol must have the opposite configuration of the hydrogen at C(1). The ready dehydration of ledol suggests the structure V for it with the hydrogen at C(1) and the hydroxyl group at C(10) *trans*- and *anti*-parallel. The hindered nature of the hydroxyl apparent in models of this structure, together with its facile dehydration, accounts for the failures to esterify ledol (Dolejš, Šorm, and Soucek 1959*a*).

The suggestion (Jefferies, Melrose, and White 1959) that palustrol (Kirjalow 1954) could be *epi*ledol, since it affords ledglycol on dehydration followed by oxidation, has been ruled out by the identification of viridiflorol as *epi*ledol (Dolejš *et al.* 1959).



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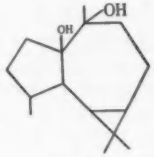


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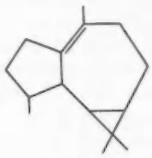

(XI)

## III. Experimental

Analyses are by Dr. K. W. Zimmermann, C.S.I.R.O. Microanalytical Laboratory, University of Melbourne. Light petroleum refers to the fraction b.p. 56–60 °C. Carbon tetrachloride and carbon disulphide solvents were used for the infra-red spectra, the latter in the region 1400–670 $cm^{-1}$. Melting points were determined in evacuated capillaries and are uncorrected. Optical rotations were observed in chloroform solutions.

Fractional distillations were carried out in a vacuum-jacketed spinning-band column of 30 theoretical plates (*n*-heptane, methyl*cyclo*hexane) using magnetic drive and incorporating a solenoid-operated automatic take off. Fractional distillations were followed by gas chromatography.

(*a*) *Sources of Aromadendrene and Globulol.**—Typical experiments were carried out on the oils of:

(i) Eucalyptus globulus. Industrial sesquiterpene residues (18 g) were filtered in light petroleum (500 ml) through silica gel (75 g). The eluate (14 g) consisted mainly of aromadendrene (I) which was identified by the comparison of its " finger-print " spectrum with that of an authentic sample. Globulol (III) was obtained by the distillation of commercial sesquiterpene alcohol residues and was recrystallized from light petroleum as needles, m.p. 87·5–88·5 °C, $[\alpha]_D^{16}$ —47° (Found: C, 81·1; H, 11·7; O, 7·2%. Calc. for $C_{15}H_{26}O$: C, 81·0; H, 11·8; O, 7·2%). The infra-red absorption spectrum showed a band at 3615 $cm^{-1}$.

(ii) E. caesia. Fractionation of oil (62 g) yielded a fraction of aromadendrene (8·2 g) (Bowyer and Jefferies 1959), b.p. 116–124 °C/10 mm, $n_D^{21}$ 1·4948, $d_4^{21}$ 0·920, $[\alpha]_D^{25}$ +11° (Found: C, 88·1; H, 11·9%. Calc. for $C_{15}H_{24}$: C, 88·2; H, 11·8%).

(iii) E. nova anglica. The commercial distillate (50 g), $[\alpha]_D^{20}$ 0°, gave eight fractions (13·8 g) with a range of constants from b.p. 108–114 °C/7 mm and $[\alpha]_D^{20}$ —18 to +9°. The fractions were essentially aromadendrene.

* The occurrence of globulol in *Eucalyptus eudesmioides* F. v. M. bark (Blumann, Michael, and White, *J. Chem. Soc.* **1953**: 788) should be amended to *E. gongylocarpa* Blakely.

(iv) Thryptomene kochii. The oil was recovered by steam distillation of the leaves and terminal branches of the plant, which was collected near Karalee, W.A., in 1958. The high boiling residue (66 g) which remained after removal of the fractions (75 g), b.p. below 74 °C/11 mm, gave four fractions (12 g), b.p. 117–130 °C/10 mm, $n_D^{18}$ 1·4986 to 1·5032, $[\alpha]_D^{20}$ +4 to +18°, which were mainly aromadendrene, and five fractions (19 g), b.p. 133–138 °C/10 mm, $d_4^{18}$ 1·5000 which were recrystallized to give globulol, m.p. 86 °C, alone, and when mixed with an authentic sample.

(*b*) *Guaiazulene.*—Globulol (III) (7·95 g) was heated with powdered sulphur (3·6 g) at 220 °C/600 mm for 3 hr. The blue azulene (2·55 g), b.p. 100–124 °C/0·5 mm, formed a trinitrobenzene complex, m.p. 150–151 °C, unchanged by admixture with an authentic sample. The samples were identical in the infra-red region 800–1300 cm$^{-1}$. The azulene was liberated from the addition complex by filtration through alumina (17 g, activity III-IV) and distilled, b.p. 118–120 °C/0·6 mm (Found: C, 90·8; H, 9·1%. Calc. for $C_{15}H_{18}$: C, 90·9; H, 9·2%). Its infra-red absorption in the "finger-print" region was identical with that of authentic guaiazulene.

The authentic samples were prepared in a similar manner, from guaiol (Ruzicka, Pontalti, and Balas 19[illegible]3).

(*c*) *Aromadendrene* (I).—(i) Globulol (III) (3·34 g) from *E. globulus* was esterified in pyridine, by standard technique to yield globulyl 3,5-dinitrobenzoate which recrystallized from light petroleum as needles, m.p. 139·5 °C, $[\alpha]_D^{20}$ −41°, with resolidification at 140 °C and again m.p. 195–197 °C (Found: C, 63·5; H, 6·7; N, 6·6%. Calc. for $C_{22}H_{26}O_6N_2$: C, 63·4; H, 6·8; N, 6·7%). Its infra-red spectrum had a peak at 1384 cm$^{-1}$. The ester (3·09 g) was heated *in vacuo* and at 140 °C decomposition occurred. Crystalline 3,5-dinitrobenzoic acid remained while the distillate, b.p. 80–81 °C/0·6 mm, $n_D^{17}$ 1·4981, $[\alpha]_D^{22}$ +14°, $d_4^{25}$ 0·9095, was collected (Found: C, 88·2; H, 11·9%. Calc. for $C_{15}H_{24}$: C, 88·2; H, 11·8%). The hydrocarbon had infra-red absorption at 3080, 1634, and 887 cm$^{-1}$ and was identified as aromadendrene (I) by comparison of its spectrum with that of an authentic sample.

(ii) Globulol (5·07 g) from *E. globulus* was dissolved in pyridine (50 ml) and treated with phosphoryl chloride (5·4 g). On the following day the mixture was poured into cold dilute hydrochloric acid and an oil (3·50 g) isolated with ether in the usual manner. The product, b.p. *c.* 80 °C/0·5 mm, was characterized as aromadendrene by its infra-red spectrum.

(iii) *epi*Globulyl 3,5-dinitrobenzoate (540 mg) was heated in the manner described above and decomposed at 165 °C. The distillate (150 mg), b.p. 80 °C/0·5 mm, $n_D^{20}$ 1·4960, $[\alpha]_D^{20}$ −10° was identified as aromadendrene by its infra-red spectrum (Found: C, 88·0; H, 12·0%. Calc. for $C_{15}H_{24}$: C, 88·2; H, 11·8%).

(*d*) apo*Aromadendrone* (II).—(i) Aromadendrene (I) was ozonized by the method described by Birch and Lahey (1953) and produced crude crystalline *apo*aromadendrone (II) (4·1 g) which after recrystallization from methanol had m.p. 83 °C, $[\alpha]_D^{20}$ +2° alone or mixed with an authentic sample. The two samples had identical "finger-print" spectra. Comparable results were obtained by ozonolysis of samples of aromadendrene derived from the oils of *E. globulus*, *E. caesia*, *E. nova anglica*, and *T. kochii* and from globulol and its 3,5-dinitrobenzoate derived from these oils. *apo*Aromadendrone had absorption at 1699 cm$^{-1}$. The semicarbazone (Briggs and Short 1928), long prisms, m.p. 192–193 °C (from methanol), was prepared in the usual manner. An orange *2,4-dinitrophenylhydrazone* recrystallized from ethyl acetate as plates, m.p. 233 °C (Found: C, 62·0; H, 6·5%. Calc. for $C_{20}H_{26}O_4N_4$: C, 62·2; H, 6·8%).

(ii) Aromadendrene (2·01 g) in ether (40 ml) was treated with osmium tetroxide (2·0 g) in ether (40 ml). After 24 hr, benzene (50 ml), ethanol (50 ml), mannitol (7 g), and KOH (7 g) in water (20 ml) were added, and the mixture refluxed for 6 hr. After addition of water the product (2·02 g), m.p. 113–114 °C, was isolated in the usual way with ether. The product (2·02 g) in ethanol (150 ml) was treated with periodic acid (9·3 g) in water (50 ml), for 12 hr. The ethanol was evaporated under reduced pressure and the product (1·1 g) filtered. Crystallization from aqueous methanol afforded long needles of *apo*aromadendrone, m.p. and mixed m.p. 82–83 °C, $[\alpha]_D^{20}$ +2°. The ketone and authentic *apo*aromadendrone had identical infra-red spectra.

(*e*) apo*Aromadendrol*.—*apo*Aromadendrone (II) (11·5 g) in ether (100 ml) was refluxed with excess lithium aluminium hydride for 1 hr. After decomposition with water and washing with dilute acid, the product (10·5 g) was recovered in the usual manner. It was distilled, b.p. 108 °C/*c.* 1 mm, and crystallized on seeding. Recrystallization gave needles, m.p. 74 °C, with hydroxyl absorption at 3629 $cm^{-1}$ (Found: C, 80·3; H, 11·7%. Calc. for $C_{14}H_{24}O$: C, 80·7; H, 11·6%). The *3,5-dinitrobenzoate* which crystallized from methanol as hair-like needles, m.p. 130–131 °C, $[\alpha]_D^{25}$ —78°, did not decompose on heating *in vacuo* to 260 °C (Found: C, 62·9; H, 6·6; N, 6·8%. Calc. for $C_{21}H_{26}O_6N_2$: C, 62·7; H, 6·5; N, 7·0%). The alcohol, m.p. and mixed m.p. 74 °C, was regenerated by refluxing the ester in 10% methanolic potassium hydroxide solution.

(*f*) *Hydrocarbon* (*VI*).—*apo*Aromadendrol (10 g) was dehydrated with phosphoryl chloride (13 g), as previously described for globulol, and yielded a hydrocarbon (VI) (8 g), b.p. 76–78 °C/1 mm, freed from chlorine by distillation from sodium (Found: C, 87·9; H, 11·8%. Calc. for $C_{14}H_{22}$: C, 88·4; H, 11·7%). The infra-red spectrum had peaks at 3070, 1670, 826, 809, and 801 $cm^{-1}$.

(*g*) apo*Aromadendrene Diol*.—Hydrocarbon (VI) (1·63 g) in ether (20 ml) was treated with osmium tetroxide (2·0 g) in ether (20 ml). One week later the reactants were worked up as described previously to yield an oily product. Crystallization from light petroleum afforded needles of apo*aromadendrene diol* (400 mg), m.p. 112–113 °C, $[\alpha]_D^{28}$ —48° (Found: C, 75·3; H, 11·0%. Calc. for $C_{14}H_{24}O_2$: C, 75·0; H, 10·8%).

(*h*) *Keto Acid* (*VII*).—(i) Prepared as described by Birch and Lahey (1953). It had absorption peaks at 1742 and 1710 $cm^{-1}$ and gave rise to their oximino acid, m.p. 167–168 °C (decomp.) (Found: C, 66·5; H, 9·2; N, 5·5%. Calc. for $C_{14}H_{23}O_3N$: C, 66·4; H, 9·1; N, 5·5%).

(ii) *apo*Aromadendrene diol (840 mg) in ethanol (100 ml) was treated with periodic acid (4·3 g) in water (50 ml). On the following day ethanol was removed under reduced pressure and on extraction, an oily residue (930 mg)—bands at 1730 and 1704 $cm^{-1}$, was obtained. The *keto-aldehyde* (900 mg) in alcohol-free acetone was oxidized with 8N chromic acid solution (1·5 ml, calc. for $CHO \rightarrow CO_2H$ 1·01 ml; *cf.* Bowden *et al.* 1946). After 30 min the keto acid (839 mg) was isolated in the usual way with ether. On treatment with hydroxylamine hydrochloride in a solution of sodium acetate in aqueous methanol, it afforded the identical oximino acid, m.p. and mixed m.p. 168–169 °C (decomp.) as described by Birch and Lahey (1953).

(iii) Crude hydrocarbon (VI) (5·3 g) in ethyl acetate was ozonized as for aromadendrene, and the product (4·4 g) in acetone was oxidized with 8N chromic acid (Bowden *et al.* 1946). Isolation of the product with ether and recovery with 10% sodium carbonate solution gave the keto acid which was identified as the oximino acid which is described above.

(*i*) *Grignard Reactions on* apo*Aromadendrone* (II).—(i) Magnesium turnings (1·2 g) and methyl iodide (9 ml) in ether (60 ml) were shaken for 30 min. To this reagent (kept at 0 °C) was added *apo*aromadendrone (6·5 g) in ether (120 ml) over a period of 45 min. The solution was refluxed for 1 hr, cooled, poured into ice-cold $NH_4Cl$ solution, and the viscous oily product (6·4 g) isolated in the usual way with ether. The product had absorption near 3600 $cm^{-1}$ but no absorption in the vicinity of 1700 $cm^{-1}$. The oil (6·4 g), 3,5-dinitrobenzoyl chloride (10 g), and pyridine (70 ml) were heated over a steam-bath for 1 hr and left overnight. Isolation in the usual way and crystallization from light petroleum gave needles of epi*globulyl 3,5-dinitrobenzoate* (3 g), m.p. 113 °C, $[\alpha]_D^{25}$ —10° (Found: C, 63·2; H, 6·7; N, 6·7%. Calc. for $C_{22}H_{28}O_6N_2$: C, 63·4; H, 6·8; N, 6·7%). The mother liquors from the above recrystallizations were chromatographed on alumina (10 g; activity II) and afforded a further crop of *epi*globulyl 3,5-dinitrobenzoate (700 mg), m.p. 113 °C, on elution with light petroleum. Ether–benzene (1 : 3) eluted an oil (20 mg) which crystallized from light petroleum as needles, m.p. 84–85 °C, alone or mixed with authentic globulol.

(ii) The above experiment was repeated with *apo*aromadendrone (4·2 g). Chromatography of the viscous product (3·95 g) on acid-washed alumina (140 g; activity I–II) provided a mobile oil (1·5 g) as the main fraction, which was eluted by 1 : 10 benzene–light petroleum and which showed no hydroxyl absorption in the infra-red. Elution with chloroform gave crude globulol (III) (900 mg), identified as the 3,5-dinitrobenzoate, m.p. and mixed m.p. 139 °C.

(*j*) epi*Globulol* (*IV*).—*epi*Globulyl 3,5-dinitrobenzoate (9 g) was dissolved in dry ether (500 ml) and lithium aluminium hydride (4·7 g) added, the reaction mixture being refluxed for 30 min. After working up in the usual way, the red viscous liquid (4 g) was thrice distilled, two fractions (1·6 and 1·2 g) being collected in the final distillation. The latter fraction had constants, b.p. 82 °C/*c*.0·4 mm, $d_4^{20}$ 0·9678, $[\alpha]_D^{20}$ —15° (Found: C, 80·8; H, 11·6%. Calc. for $C_{15}H_{26}O$: C, 81·0; H, 11·8%). It had hydroxyl absorption at 3620 $cm^{-1}$.

(*k*) *Dehydration of* epi*Globulol* (*IV*).—*epi*Globulol (2·65 g) was heated over a steam-bath for 20 min in acetone (75 ml), water (10 ml), and conc. $H_2SO_4$ (5 ml). After dilution with water and extraction with ether in the usual way the oily product (2·42 g) was dissolved in light petroleum and filtered through silica gel (16 g). The eluate (2·30 g) had no hydroxyl absorption in the region of 3600 $cm^{-1}$.

(*l*) *Ledglycol* (*IX*).—(i) The above product (1·8 g) was suspended in acetone (50 ml) and water (25 ml) while potassium permanganate (2·3 g) was added portion-wise with stirring—the colour disappearing after each addition. The solution was decanted from manganese dioxide, diluted with water, and extracted with ether. The product (400 mg) was purified by treatment with Girard's reagent (cf. Beereboom, Fazakerley, and Halsall 1957), and chromatography on alumina (10 g; activity II). After elution with light petroleum–benzene (250 ml; 1 : 1) the column was exhausted with chloroform to yield an eluate (109 mg) which after two recrystallizations from light petroleum had m.p. 151–152 °C alone or mixed with authentic ledglycol (IX) (Birch *et al.* 1959). The two compounds had identical infra-red spectra.

(ii) The hydrocarbon (890 mg) from the dehydration of *epi*globulol was dissolved in dry ether (50 ml) and treated in the dark with osmium tetroxide (1 g). After 11 days the reactants were diluted with dry ether (150 ml) and refluxed with excess lithium aluminium hydride for 1½ hr. The product (875 mg) was worked up by the standard method and chromatographed on alumina (20 g; activity II). The fraction (480 mg) which was eluted by chloroform, had m.p. and mixed m.p. 150–152 °C with authentic ledglycol (IX) after two recrystallizations from light petroleum. "Finger-print" spectra confirmed their identity.

## IV. Acknowledgments

The authors wish to record their thanks to Dr. A. R. H. Cole for the interpretation of some of the infra-red spectra; to Dr. A. Blumann of Messrs. Plaimar Ltd., Western Australia, who distilled the *Eucalyptus globulus* oil residues kindly supplied by Don Ramón Bordas of Seville, Spain; to Professor F. N. Lahey for samples of aromadendrene and *apo*aromadendrone; to Professor A. J. Birch for a sample of ledglycol; and to the Government Botanist for identification of plant material.

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# THE CHEMICAL CONSTITUENTS OF AUSTRALIAN *FLINDERSIA* SPECIES

## XI. THE STRUCTURES OF MACULOSIDINE AND MACULOSINE, TWO ALKALOIDS FROM F. MACULOSA LINDL.

By R. H. Prager,* E. Ritchie,* and W. C. Taylor*

[*Manuscript received March* 18, 1960]

*Summary*

Maculosidine has the structure I, previously suggested. By degradation it is converted to 6,8-dimethoxy-3-ethyl-4-hydroxy-2-quinolone, identified by synthesis.

Maculosine, which has formula $C_{17}H_{17}O_6N$ and the typical ultraviolet spectrum of a furoquinoline, is oxidized by periodate to a substance which is degraded to the known 3-ethyl-4-hydroxy-6,7-methylenedioxy-2-quinolone. This evidence leads to the assignment of IV as its structure.

### I. Maculosidine

On the grounds that maculosidine, a dimethoxydictamnine, was not identical with kokusaginine or skimmianine and that it did not contain a methoxyl group at the 5-position since *iso*maculosidine was not readily demethylated, Brown *et al.* (1954) assigned to it structure I. This negative evidence was somewhat weakened by the fact that *iso*maculosidine was obtained only as a hemihydrate, but further work was precluded by lack of material. More material has now been accumulated and the problem resumed.

Firstly, it was established that *iso*maculosidine retained water of crystallization very tenaciously. Prolonged drying at 120 °C/1 mm was necessary to obtain an anhydrous specimen. Moreover, since *iso*maculosidine rapidly assumed the pink colour characteristic of *iso*furoquinolines there was no doubt that it was a member of this class.

Degradation of the alkaloid was accomplished by the method used for maculine (Gell, Hughes, and Ritchie 1955*b*). Hydrogenolysis in the presence of Adams's catalyst yielded a substance, $C_{14}H_{17}O_4N$, which was easily demethylated by acid to a substance, $C_{13}H_{15}O_4N$, readily recognized as a 3-ethyl-4-hydroxy-2-quinolone derivative. 6,8-Dimethoxy-3-ethyl-4-hydroxy-2-quinolone was synthesized from 2,4-dimethoxyaniline and diethyl ethylmalonate in boiling diphenyl ether and was identical with the product obtained from maculosidine as shown by direct comparison (mixed melting points and infra-red spectra) of the substances themselves and their acetyl derivatives.

The substance, $C_{14}H_{17}O_4N$, obtained from the hydrogenolysis of maculosidine showed in the ultraviolet a maximum at 280 mμ (Fig. 1) and in the infra-red a band at 1646 $cm^{-1}$, and is therefore assigned structure II. This assignment is made on the basis of published spectral data: thus, Grundon, McCorkindale,

* Department of Organic Chemistry, University of Sydney.

and Roger (1955) have shown that in the ultraviolet 2-quinolones have a band at 280 mμ, not shown by 4-quinelones, and furthermore Price and Willis (1959) have found, in clarifying this work, that in the infra-red the carbonyl absorption of 4-quinolones usually occurs at distinctly lower frequencies, although the range is 1618–1647 $cm^{-1}$. It follows that maculosidine is I.

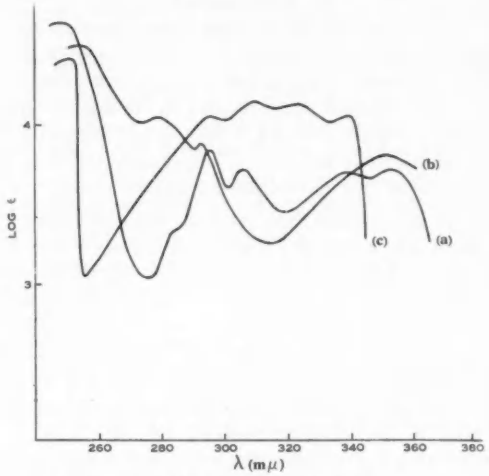
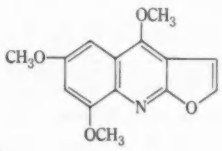
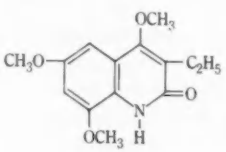



Fig. 1.—Ultraviolet spectra in ethanol.
(*a*) Maculosidine.
(*b*) Hydrogenolysis product of maculosidine.
(*c*) Maculosine.

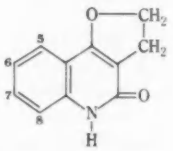
The ultraviolet spectra of most furoquinolone alkaloids show a broad envelope extending from about 260 to 350 mμ and having four more or less distinct peaks. The spectrum of maculosidine differs in two ways: (i) the envelope extends to about 370 mμ and (ii) it has a well-defined minimum at about 315 mμ (Fig. 1).




(I)




(II)




(III)

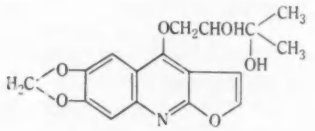
These apparent anomalies are explicable on the basis of the observation by Sato and Ohta (1958), who found that whereas a methoxyl group at the 6-position in III displaces the Lb band (nomenclature of Platt 1949) to longer wavelengths but scarcely affects the La band, a methoxyl group at the 8-position has little effect on the $\lambda_{max.}$ of either the La or Lb band. In the present instance these

effects would be expected to produce a broader envelope and a marked minimum at approximately the wavelengths observed. However, it is curious that these effects are not obvious in the spectrum of the hydrogenolysis product II (Fig. 1), which structurally resembles more closely the compounds studied by Sato and Ohta (1958).

## II. Maculosine

Brown *et al.* (1954) assigned to maculosine the formula $C_{15}H_{15}O_5N$, noted that it gave a positive Labat test for a methylenedioxy group, but no blue colour on warming with concentrated sulphuric acid as other methylenedioxy-containing furoquinolines do, and indicated that although a methoxyl group was absent (the small value found being evidently due to an impurity) the ultraviolet spectrum was very similar to that of a typical furoquinoline alkaloid (Fig. 1). It has now been found that further extensive purification removes the impurity responsible for the methoxyl value and that the analyses are in better agreement with the formula $C_{17}H_{17}O_6N$. A methylimino group is absent but the presence of a methylenedioxy group is confirmed by positive tests with the Labat reagent and with chromatropic acid (Hansen 1953), the production of a bluish green colour with warm concentrated sulphuric acid, and the presence of a double peak of low intensity at 920 and 935 $cm^{-1}$ in the infra-red spectrum (Briggs *et al.* 1957). Also, maculosine is optically active, having $[\alpha]_D^{20}$ +36° (dry pyridine).

Maculosine showed no phenolic properties and as the infra-red spectrum showed a broad band at 3140–3350 $cm^{-1}$ and peaks at 1080 and 1030 $cm^{-1}$, the presence of alcoholic hydroxyl groups was indicated. All these data suggested that maculosine might be represented by IV, and this was confirmed by degradation.




(IV)

On oxidation with periodate, maculosine consumed more than 1 mole (1·25 and 1·4) yielding acetone and formaldehyde together with a product, which on hydrogenolysis yielded the known 3-ethyl-4-hydroxy-6,7-methylenedioxy-2-quinolone (Gell, Hughes, and Ritchie 1955*b*). Under similar conditions evoxine (Eastwood, Hughes, and Ritchie 1954) and evolatine (Gell, Hughes, and Ritchie 1955*a*) also yielded formaldehyde and acetone. Presumably, the formaldehyde arose by periodate oxidation of glycollic aldehyde formed by cleavage of the initially produced aryloxyacetaldehyde.

3-Ethyl-4-hydroxy-6,7-methylenedioxy-2-quinolone was also obtained from maculosine by the reactions used to degrade maculosidine. Hydrogenolysis yielded a tetrahydro derivative from which hot acid removed the isoprenoid residue affording the 2-quinolone.

## III. Experimental

Melting points are uncorrected. Light petroleum refers to the fraction of b.p. 60–90 °C. Ultraviolet spectra were measured in purified ethanol on a Hilger " Uvispek ". Infra-red spectra of substances in a paraffin mull were recorded on a Perkin–Elmer Infracord 137. Analyses were performed by Miss B. Stevenson of these Laboratories, and by the C.S.I.R.O. Microanalytical Laboratories, Melbourne.

(*a*) *Maculosidine.*—Light absorption : ultraviolet, $\lambda_{max.}$ 249, 284 (shoulder), 295, 305, 340, and 353 mμ ; log ε 4·66, 3·33, 3·85, 3·72, 3·70, and 3·72 respectively.

(*b*) iso*Maculosidine.*—The substance crystallized from benzene in colourless needles, m.p. 168 °C, which soon assumed a characteristic pink colour (Found (on material dried at 50 °C/1 mm for 12 hr) : C, 58·4 ; H, 5·7 ; $OCH_3$, 21·1%. Calc. for $C_{14}H_{13}O_4N.1\frac{1}{2}HO_2$ : C, 58·9 ; H, 5·6 ; $OCH_3$, 21·7%. Found (on material dried at 120 °C/1 mm for 50 hr) : C, 64·3 ; H, 5·0%. Calc. for $C_{14}H_{13}O_4N$ : C, 64·9 ; H, 5·1%). Light absorption : infra-red, 1600, 1557, and 1510 $cm^{-1}$.

(*c*) *Hydrogenolysis of Maculosidine.*—A solution of maculosidine (0·2 g) in ethanol (50 ml) was shaken in an atmosphere of hydrogen in the presence of Adams's catalyst. When hydrogen uptake had ceased, the filtered solution was evaporated to dryness, and the residue recrystallized from light petroleum. The *product II* (0·15 g) formed colourless needles, m.p. 126–127 °C (Found : C, 63·7 ; H, 6·4%. Calc. for $C_{14}H_{17}O_4N$ : C, 63·9 ; H, 6·5%). Light absorption : ultraviolet, $\lambda_{max.}$ 255, 280, 292, and 350 mμ ; log ε 4·51, 4·05, 3·88, and 3·82 respectively ; infra-red, 1646, 1618, and 1508 $cm^{-1}$.

(*d*) *6,8-Dimethoxy-3-ethyl-4-hydroxy-2-quinolone.*—Demethylation of the above substance (0·1 g) was effected by heating it under reflux with 5N hydrochloric acid (15 ml) for 0·5 hr. After cooling and diluting, the product was collected, dissolved in dilute sodium hydroxide, and reprecipitated by acetic acid. The *substance* crystallized from ethanol as colourless needles, m.p. 293–294 °C (Found : C, 62·5 ; H, 6·1%. Calc. for $C_{13}H_{15}O_4N$ : C, 62·6 ; H, 6·1%).

The *acetyl derivative*, prepared by heating the substance with acetic anhydride and pyridine on the steam-bath, crystallized from benzene in colourless needles, m.p. 193–194 °C (Found : C, 61·9 ; H, 5·9%. Calc. for $C_{15}H_{17}O_5N$ : C, 61·9 ; H, 5·9%).

(*e*) *Synthesis of 6,8-Dimethoxy-3-ethyl-4-hydroxy-2-quinolone.*—A solution of 2,4-dimethoxyaniline (1·4 g) and diethyl ethylmalonate (1·9 g) in diphenyl ether (20 ml) was vigorously boiled under reflux for 0·75 hr and then allowed to cool. The crystalline product (0·65 g) which separated, was recrystallized from ethanol as colourless needles, m.p. 293–294 °C (Found : C, 62·6 ; H, 6·2%. Calc. for $C_{13}H_{15}O_4N$ : C, 62·6 ; H, 6·1%). The m.p. was undepressed by admixture with the above degradation product and the infra-red spectra were identical.

The acetyl derivative, m.p. 193–194 °C (Found : C, 62·2 ; H, 5·9%. Calc. for $C_{15}H_{17}O_5N$ : C, 61·9 ; H, 5·9%) was shown by similar means to be identical with the above acetyl derivative.

(*f*) *Maculosine.*—The alkaloid, further purified by chromatography and recrystallization, had m.p. 229–230 °C, $[\alpha]_D^{25}$ +36° (*c*, 0·83 in dry pyridine) (Found : C, 61·6, 61·6, 61·5 ; H, 5·3, 5·2, 5·4 ; O, 28·9 ; N, 4·7% ; $(N)CH_3$, nil ; $OCH_3$, nil. Calc. for $C_{17}H_{17}O_6N$ : C, 61·6 ; H, 5·2 ; O, 29·0 ; N, 4·2%). Light absorption : ultraviolet, $\lambda_{max.}$ 252, 295, 310, 323, and 340 mμ ; log ε 4·42, 4·05, 4·16, 4·15, and 4·06 respectively ; infra-red, 3140–3350, 1080, 1030, 920, and 935 $cm^{-1}$.

(*g*) *Degradation of Maculosine.*—(i) A solution of the alkaloid (0·03 g) in pure dioxan (20 ml) was treated with excess aqueous 0·1N periodic acid and kept at room temperature for 2 days. In duplicate experiments, titration of aliquots showed that 1·25 and 1·4 moles respectively had been consumed. Excess reagent was reduced with sodium arsenite, the mixture made 5N with respect to hydrochloric acid, and steam distilled, the distillate being collected in an aqueous acidic 2,4-dinitrophenylhydrazine reagent.

The solution remaining after steam distillation was basified with ammonia, saturated with salt, and then continuously extracted with chloroform. The extract was dried, evaporated, and the residue hydrogenolysed in the usual manner. The product (0·01 g) crystallized from ethanol in yellow needles which were further purified by sublimation at 222 °C/$10^{-4}$ mm to yield colourless

needles (0·007 g), m.p. 301–303 °C, undepressed by admixture with an authentic specimen of 3-ethyl-4-hydroxy-6,7-methylenedioxy-2-quinolone (Found: C, 61·8; H, 5·1%. Calc. for $C_{12}H_{11}O_4N$: C, 61·8; H, 4·7%). The infra-red spectra of the two samples were identical.

The precipitate, which had formed in the DNP reagent, was purified by passing its benzene solution through a short colume of acid-washed alumina. On paper chromatography using the method of Gasparic and Vacera (1957), this material showed two spots indistinguishable from those given by acetone and formaldehyde DNP's ($R_F$ 0·46 and 0·15 respectively). The two substances were isolated by fractional crystallization from benzene and *cyclo*hexane and their identities confirmed by mixed m.p.'s and infra-red spectra.

(ii) On hydrogenolysis by the usual method maculosine (0·05 g) took up 8·0 ml of hydrogen (Calc. for 2 moles: 8·3 ml). The product crystallized from benzene–light petroleum as colourless needles (0·04 g), m.p. 202–203 °C, but was not obtained in pure form (Found: C, 59·4; H, 6·2%. Calc. for $C_{17}H_{31}O_6N$: C, 60·9; H, 6·3%). It was hydrolysed by heating under reflux with 5N hydrochloric acid (5 ml) for 0·5 hr. After cooling, the precipitate was collected, washed thoroughly with aqueous sodium acetate and water, dried, and recrystallized from ethanol as colourless needles, m.p. 302–304 °C. It was identified as 3-ethyl-4-hydroxy-6,7-methylenedioxy-2-quinolone by mixed m.p. and infra-red spectrum.

## IV. Acknowledgment

The authors are grateful to C.S.I.R.O. for the award of a scholarship to one of them (R.H.P.).

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# CHEMICAL STUDIES OF THE MYRTACEAE

## II. THE CONSTITUENTS OF SYNCARPIA LAURIFOLIA TENN.*

By DIANA HODGSON,† E. RITCHIE,† and W. C. TAYLOR†

[*Manuscript received March* 31, 1960]

*Summary*

The bark of *Syncarpia laurifolia* Tenn. yields β-sitosterol, betulinic acid, ursolic acid, and "syncarpic acid" which is identified as 1,1,5,5-tetramethyl*cyclo*hexan-2,4,6-trione (I). Some reactions of this substance are described.

## I. INTRODUCTION

*Syncarpia laurifolia* Tenn., family Myrtaceae, is commonly known as turpentine tree, and is widely distributed throughout the coastal regions of New South Wales. The timber is used extensively for piles, being somewhat resistant to marine borers. In a previous investigation of the bark Ralph and White (1949) isolated betulinic acid in minute yield from an alcoholic extract. In the present work light petroleum and ether extracts afforded large acidic fractions from which were separated by usual methods β-sitosterol (0·08 per cent.), betulinic acid (1·2 per cent.), ursolic acid (0·6 per cent.), and a third acidic compound (0·7 per cent.) which for convenience is referred to as "syncarpic acid".

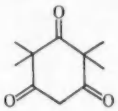
Syncarpic acid, $C_{10}H_{14}O_3$, was identified as the known compound, 1,1,5,5-tetramethyl*cyclo*hexan-2,4,6-trione (I) by direct comparison with authentic material prepared by acid hydrolysis of the tetramethyl derivative (III) of phloroacetophenone (Riedl and Risse 1954). Thus the acidity of the substance, which exceeds that of acetic acid ($pK_a$'s 4·1 and 4·7 respectively), is due to the existence of the enolic form (IIa). *O*-Methyl (IIb), *O*-acetyl (IIc), and *C*-acetyl (III) derivatives were prepared by standard methods. Oxidation with alkaline hydrogen peroxide gave the expected product, di*iso*propyl ketone. Syncarpic acid reacted readily with formaldehyde to give a methylene derivative.

Clearly, the enolized β-diketone system of syncarpic acid is incapable of forming metal chelate complexes, being "*trans*fixed", but since the reaction of enols with ferric ion does not depend on chelation (Milburn 1955) syncarpic acid, like dimedone (IV), gives a strong *wine-red* colour with aqueous ferric chloride solution; in ethanolic solutions the colouration is less distinctive. Thus Chan and Hassall (1955) have argued incorrectly that *cyclo*hexan-1,3-diones should not give a ferric colour; alcoholic solutions were used, although it is known (Wesp and Brode 1934) that aqueous solutions give maximum colour intensity.

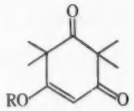
* Part I of this series is regarded as: The constituents of the kino of *Eucalyptus maculata* Hook. See *Aust. J. Chem.* **11**: 372 (1958).

† Department of Organic Chemistry, University of Sydney.

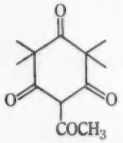
The ultraviolet absorption spectrum of syncarpic acid is in keeping with the assigned structure, being pH-dependent in a fashion characteristic of *cyclo*hexan-1,3-diones (Meek, Turnbull, and Wilson 1953). At pH 3 the compound showed $\lambda_{max.}$ 258 mμ (ε, 16,700) and at pH 10 $\lambda_{max.}$ 280 mμ (ε, 28,000) corresponding to 100 per cent. enol and enol anion, respectively; even in "neutral 95 per cent. ethanol" purified in the usual way the compound showed $\lambda_{max.}$ 280 mμ (ε, 28,000).



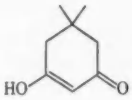
(I)



(IIa, R=H)
(IIb, R=Me)
(IIc, R=$COCH_3$)

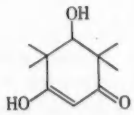


(III)

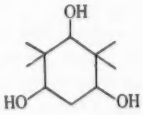


(IV)

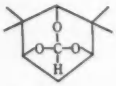
Catalytic hydrogenation of syncarpic acid was unsatisfactory, but reduction with lithium in liquid ammonia readily gave the dihydro derivative (V) (also prepared by sodium borohydride reduction of IIb), while reduction in the presence of ethanol produced the hexahydro derivative (VI). In this compound the three hydroxyl groups were shown to be *cis* by the ready formation of the orthoformate (VII) (Stetter and Steinacker 1953). It is worth noting that this represents a *meso*-form; if two hydroxyl groups were *cis* and the third *trans* placed, two other stereoisomers become possible, a DL- and a second *meso*-form.



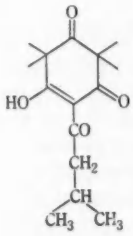
(V)



(VI)



(VII)



(VIII)

Syncarpic acid is the first simple *C*-methylated derivative of phloroglucinol to be isolated from natural sources. In view of the common occurrence of related *C*-acylated derivatives in the Myrtaceae family (Birch and Elliot 1956) the possibility exists that the acid is an artefact derived during working up from a related *C*-acyl compound such as leptospermone (VIII). This is considered unlikely because of the mildness of the extraction and particularly in view of the known stability of such compounds to alkaline hydrolysis (Riedl and Risse 1954). Thus, III was recovered unchanged after 8 hr refluxing in aqueous sodium hydroxide solution. Nevertheless, isolation of most of the compound I by saponification of the neutral fraction of the plant extract does suggest the presence of some precursor, which could be an *O*-alkyl or *O*-acyl derivative.

## II. Experimental

Melting points are uncorrected. Light petroleum refers to the fraction of b.p. 60–90 °C. Ultraviolet spectra were measured in purified ethanol on a Hilger Uvispek. Infra-red spectra of substances in paraffin mulls were recorded on a Perkin-Elmer Infracord 137. Analyses were performed by Miss B. Stevenson of these laboratories, and by the C.S.I.R.O. Microanalytical Laboratories, Melbourne.

(*a*) *Extraction of the Bark.*—The milled bark (6·7 kg) was exhausted by cold percolation with light petroleum followed by ether. The concentrated light petroleum extract (600 g: this and other weights of crude fractions are only approximate because of the difficulty of drying), a red-brown gum, was dissolved in ether (6 l.), the solution filtered (a small amount of waxy material was separated and discarded) and then extracted with 5% $NaHCO_3$ (5×200 ml) to give only a trace of acidic material, which was discarded. Because of the extensive overlap of material which occurred in the subsequent work, all aqueous alkaline extracts were thoroughly back-extracted with ether, and all acidic fractions were purified by recycling at appropriate stages. In this way extraction of the ether solution of the concentrate with 5% sodium carbonate (5×200 ml) gave a gummy semicrystalline acid (*B*) (4·5 g). Extraction of the ether solution next with 2% NaOH (10×400 ml) followed by 8% NaOH (3×200 ml) gave combined material of three layers: the upper ethereal layer containing now essentially neutral material (A), a middle layer (C) consisting of crude triterpene acid salts, and a clear aqueous bottom layer (D). The middle layer, C, was recycled between ether and 5% NaOH (1·5 l.) to give upper and lower layers which were added to fractions A and D respectively, and a middle layer which yielded an acid fraction ($C_1$) (140 g), a red-brown jelly. Fraction D gave acidic material which was combined with fraction B; crystallization from benzene gave "syncarpic acid" (8·0 g); further amounts were obtained by extracting the gummy mother liquors with 2% sodium hydroxide (total yield 15·8 g).

Fraction $C_1$ could not be crystallized directly, nor could a crystalline sodium salt be obtained with aqueous ethanolic NaOH. Considerable purification was achieved initially by prolonged boiling of the sodium salts in ethanol in the following manner. A solution of $C_1$ and NaOH (100 g) in ethanol (600 ml) and water (400 ml) was refluxed for 3 hr, and then boiled down to about one half-bulk. An equal volume of water was added and the mixture kept overnight. The dark brown supernatant liquid was decanted from a dark semisolid precipitate which was taken up in hot ethanol (300 ml); this solution was treated with hot 10% NaOH (200 ml) and the mixture concentrated until oily drops appeared. After cooling and keeping overnight the gum which separated was washed with ether and then shaken with dilute acid and chloroform (2 l.). The lower layer was separated and filtered from a fine solid ($E_1$). The chloroform was removed from the filtrate, the residue dissolved in a large volume of ether, and the solution concentrated to about 600 ml, giving a solid ($E_2$): the filtrate was concentrated to 100 ml, giving a jelly from which a solid ($E_3$) was obtained on dissolution in ether. $E_1$, $E_2$, and $E_3$ were combined and crystallized from methanol; by working up the mother liquors there was eventually obtained 24·4 g of colourless needles, m.p. 274–276 °C. The pure substance (from ethanol) had m.p. 279–280 °C, undepressed on admixture with authentic ursolic acid, m.p. 280–281 °C (Found: C, 78·5; H, 10·8%. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%). The infra-red spectra were identical; the m.p. and mixed m.p. of the methyl esters were 170–171 °C.

The total neutral fraction A was saponified by refluxing for 3 hr with 10% aqueous ethanolic NaOH (2 l.) and the ethanol then distilled off. Water (2·5 l.) was added and the mixture shaken with ether. This produced three layers each of which after back-extraction, recycling, and recombination of appropriate solutions, gave three partially purified fractions. The upper ether layer containing neutral material (141 g) was steam distilled (trace of volatile material discarded) and the residue allowed to stand firstly in ether (400 ml) and then in light petroleum (3 l.); material which separated during this treatment was further purified by boiling with ether, giving eventually ursolic acid (3·3 g). This is an excellent example of the carryover of material which constantly occurred. The light petroleum-soluble material (113 g) was chromatographed on a large column of alumina; material eluted by light petroleum, benzene–light petroleum, and benzene (58 g) and by ether–methanol and methanol (15 g) did not crystallize on further chromatography or on

vacuum distillation. Material eluted by benzene–ether and ether (25 g) was rechromatographed on alumina to give β-sitosterol (2·9 g), m.p. and mixed m.p. 135–136 °C.

The middle layer was processed in a similar manner to that described above for fraction C, to yield after repeated purification ursolic acid (11·1 g). Methyl ursolate (3·1 g) was obtained by methylation of the residues and chromatography of the crude esters on alumina ; this is equivalent to a total yield of acid of 41·9 g (0·6%). The clear lower layer afforded an acidic fraction (100 g) which on stirring with a little benzene yielded syncarpic acid (26 g). The non-crystallizable material in ethereal solution was fractionated with alkali to give fractions which were recycled to remove neutral material persistently carried over ; in this way a further 3·2 g of syncarpic acid was separated. The residues (43·4 g) did not yield any homogeneous fraction on chromatography or distillation of the methylated material.

The concentrated ether extract (200 g) was a light brown friable solid. An ethereal solution (10 l.) was shaken thoroughly with 5% NaOH (4 l.). The voluminous precipitate of sodium salts which formed was collected and purified by boiling with 10% aqueous ethanolic NaOH, giving eventually, after recrystallization, a nearly colourless sodium salt (91 g). On decomposition this gave betulinic acid (81 g), needles from methanol, m.p. 315–317 °C, undepressed on admixture with an authentic specimen (Found : C, 78·5 ; H, 10·5%. Calc. for $C_{30}H_{48}O_3$ : C, 78·9 ; H, 10·6%). The infra-red spectrum was identical with that of authentic betulinic acid. No further crystalline material was obtained from the alkaline filtrates. The neutral fraction (80 g) was saponified by refluxing for 3 hr in aqueous ethanolic 10% NaOH (500 ml). The neutral fraction from this on chromatography gave β-sitosterol (2·3 g) making a total of 5·2 g (0·08%) ; a small middle layer of sodium salts was intractable but from the aqueous lower layer was separated a further 3·2 g of syncarpic acid, making a total of 46 g (0·7%).

(*b*) *Syncarpic Acid.*—This crystallized with great ease from benzene in colourless plates, m.p. 190 °C, with sintering and change of crystalline habit at about 175 °C, undepressed on admixture with an authentic specimen prepared by the method of Riedl and Risse (1954) (Found : C, 66·0 ; H, 7·7 ; O, 26·3%. Calc. for $C_{19}H_{14}O_3$ : C, 65·9 ; H, 7·7 ; O, 26·4%) ; $pK_a$ (in water), 4·1. Light absorption : at pH 3, $\lambda_{max.}$ 258 mμ (ε, 16,700), and at pH 10, $\lambda_{max.}$ 280 mμ (ε, 28,000) ; bands in the infra-red at 2720–2560, 1710, 1615, and 1540 $cm^{-1}$. Syncarpic acid gave a moderately strong red-brown colour with ethanolic ferric chloride ; when a solution in a few drops of ethanol was diluted with water and treated with aqueous ferric chloride a strong wine-red colour was produced. Quantitatively, equal volumes of freshly prepared 0·02M aqueous solutions of syncarpic acid (dissolved first in a minimum volume of ethanol) and ferric chloride (pH 2·5) on mixing gave a solution with $\lambda_{max.}$ 490–500 mμ and optical density 1·04 ; similarly, dimedone gave a solution with $\lambda_{max.}$ 490–500 mμ, optical density 0·43, and *m*-nitrophenol gave a solution with $\lambda_{max.}$ 510 mμ and optical density 0·41.

The O-*methyl derivative* (*IIb*), prepared with diazomethane, crystallized from light petroleum in needles, m.p. 63 °C (Found : C, 67·3 ; H, 8·2 ; OMe, 14·2%. Calc. for $C_{11}H_{16}O_3$ : C, 67·3 ; H, 8·2 ; 1×OMe, 15·8%). Light absorption : $\lambda_{max.}$ 258 mμ (ε, 17,800) and bands in the infra-red at 1710, 1645, and 1615 $cm^{-1}$.

The O-*acetate* (*IIc*), prepared in acetic anhydride–pyridine at room temperature, crystallized from light petroleum in needles, m.p. 49 °C (Found : C, 63·8 ; H, 7·1%. Calc. for $C_{12}H_{16}O_4$ : C, 64·3 ; H, 7·2%. Light absorption : $\lambda_{max.}$ (in hexane) 227 mμ (ε, 8,600) and bands in the infra-red at 1770, 1720, 1665, and 1640 $cm^{-1}$.

The *C*-acetyl derivative (III) was prepared by heating syncarpic acid with acetic anhydride–sodium acetate (Chan and Hassall 1955). It crystallized from aqueous methanol as prisms, m.p. 54 °C, undepressed on admixture with an authentic specimen (Found : C, 64·0 ; H, 7·0%. Calc. for $C_{12}H_{16}O_4$ : C, 64·3 ; H, 7·2%). Syncarpic acid, on warming in methanol solution with formalin, gave the *methylenebis-derivative*, prisms from aqueous ethanol, m.p. 171 °C (Found : C, 66·9 ; H, 7·4%. Calc. for $C_{21}H_{28}O_6$ : C, 67·0 ; H, 7·5%).

(*c*) *Oxidation of Syncarpic Acid.*—A mixture of syncarpic acid and hydrogen peroxide (5 ml of 100 vol) in pyridine (10 ml) was allowed to stand for 24 hr and then poured with cooling into hydrochloric acid. Work-up with ether in the usual way gave a neutral fraction, b.p. 120 °C,

which was identified as di*iso*propyl ketone by means of the semicarbazone and dinitrophenylhydrazone derivatives which had m.p. and mixed m.p. with authentic specimens, 158 and 96 °C respectively.

(*d*) *Dihydrosyncarpic Acid* (*V*).—(i) To a solution of syncarpic acid (1 g) in liquid ammonia (80 ml) was added lithium (200 mg) with stirring over a period of 20 min, when excess lithium was destroyed with ethanol. Work-up in the usual way gave an *acidic product*, needles from benzene–ethanol, m.p. 197 °C (Found: C, 65·0; H, 8·7%. Calc. for $C_{10}H_{16}O_3$: C, 65·2; H, 8·7%). Light absorption: $\lambda_{max.}$ 280 mμ (ε, 24,000) and bands in the infra-red at 3320, 2710–2560, 1600, 1525, and 1500 $cm^{-1}$.

The O-*methyl derivative* crystallized from light petroleum in prisms, m.p. 128 °C (Found: C, 66·6; H, 8·9%. Calc. for $C_{11}H_{18}O_3$: C, 66·6; H, 9·1%). Light absorption: $\lambda_{max.}$ 240 mμ (ε, 13,250) and bands in the infra-red at 3320, 1655, 1605, and 1595 $cm^{-1}$.

(ii) *O*-Methylsyncarpic acid (580 mg) in methanol (5 ml) was treated with sodium borohydride (100 mg) in water (5 ml); the solution was allowed to stand for 1 hr, 0·2N NaOH (5 ml) added and the mixture warmed for 30 min on the steam-bath. On cooling, the acidified solution was extracted with ether to yield the dihydro derivative (435 mg).

(*e*) *Hexahydrosyncarpic Acid* (*VI*).—A solution of syncarpic acid (1 g) in ether (30 ml) and ethanol (3 ml) was added slowly with stirring to a solution of lithium (200 mg) in liquid $NH_3$ (60 ml). Immediate reaction occurred and after a few minutes the ammonia was allowed to evaporate. The residue was taken up in dilute acid, the solution washed with ether, saturated with $NH_4SO_4$, and reextracted with ether to give the *hexahydro derivative* (*VI*), prisms from benzene–ethanol, m.p. 200 °C (Found: C, 63·4; H, 10·6%. Calc. for $C_{10}H_{20}O_3$: C, 63·8; H, 10·7%).

The *triacetate*, prepared with acetic anhydride–pyridine at room temperature, crystallized from light petroleum in needles, m.p. 95 °C (Found: C, 61·1; H, 8·6%. Calc. for $C_{16}H_{26}O_6$: C, 61·1; H, 8·3%).

(*f*) *Hexahydrosyncarpic Acid Orthoformate* (*VII*).—Freshly dried VI (540 mg) and ethyl orthoformate (2·5 g) were mixed in absolute methanol (4·3 ml) containing hydrogen chloride (43 mg). The solution was allowed to stand for 3 days, and then neutralized with potassium carbonate and the methanol removed *in vacuo*. The residue was extracted with ether to give the *orthoformate* (*VII*) as the soluble fraction, crystallizing in prisms from light petroleum, m.p. 59–60 °C (Found: C, 66·8; H, 9·2%. Calc. for $C_{11}H_{18}O_3$: C, 66·6; H, 9·2%). There were no hydroxyl bands in the infra-red spectrum.

## III. Acknowledgments

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# THE CHEMISTRY OF EUCALYPT KINOS

## III. (+)-AFZELECHIN, PYROGALLOL, AND (+)-CATECHIN FROM EUCALYPTUS CALOPHYLLA KINO

By W. E. Hillis* and Ann Carle*

[*Manuscript received March* 31, 1960]

*Summary*

Pyrogallol, (+)-catechin, and a new flavanol, (+)-afzelechin (3,5,7,4′-tetrahydroxyflavan), have been isolated from the kino of *Eucalyptus calophylla* R.Br. Gallocatechin and *epi*catechin have been identified chromatographically. In addition, a compound which resembles (−)-*epi*afzelechin chromatographically, has been detected.

### I. Introduction

Aromadendrin, kaempferol, and ellagic acid have been isolated previously from *Eucalyptus calophylla* R.Br. kino (see Part II of this series, Hillis 1952). Recently, when fresh samples of this kino were examined by two-dimensional paper chromatography, a number of other components with the colour reaction of flavanols containing a phloroglucinol A ring, were also revealed. Three of these flavanols—6, 9, 11 (see Fig. 1)—were chromatographically indistinguishable from (+)-gallocatechin, (−)-*epi*catechin, and (+)-catechin respectively when co-chromatographed on a two-dimensional chromatogram. The identity of (+)-catechin has been confirmed by other methods.

The chromatographic properties of component 14 in *n*-butanol : acetic acid : water (6 : 1 : 2, B.A.W.) differ from those of (+)-catechin ($\Delta R_M = -0 \cdot 35$) in an amount similar to the difference between (+)-catechin and (+)-gallocatechin ($\Delta R_M = +0 \cdot 39$, Table 1). The relationship between (−)-*epi*afzelechin (King, Clark-Lewis, and Forbes 1955) and component 14 ($\Delta R_M = 0 \cdot 20$) is similar to that between (−)-*epi*catechin and (+)-catechin ($\Delta R_M = 0 \cdot 22$), and (−)-*epi*-gallocatechin and (+)-gallocatechin ($\Delta R_M = 0 \cdot 21$). Bradfield and Bate-Smith (1950) have reported very similar $\Delta R_M$ values for the catechins and the gallocatechins. Component 14 also possessed an $R_F$ value in 6 per cent. acetic acid that was similar to those of the other flavanols (Table 1). The chromatographic properties of component 14 suggest that it is a member of the flavanol series.

In a chemical examination of component 14, a tetra-acetate was prepared and in addition an alkali-insoluble trimethyl ether and an acetate of the latter. Oxidation of the trimethyl ether with potassium permanganate in acetone yielded *p*-anisic acid. The addition of vanillin–hydrochloric acid to the component produced an immediate red colour identical with those given by the other flavanols. This colour reaction shows the presence of a phloroglucinol nucleus

* Division of Forest Products, C.S.I.R.O., Melbourne.

in a flavanol (Swain and Hillis 1959). The properties of the component are consistent with those of a 3,5,7,4′-tetrahydroxyflavan. The specific rotation of +20° is of the same order as +17° for (+)-catechin (Freudenberg 1933) and 15° for (+)-gallocatechin (Mayer 1956). Consequently component 14 has been named (+)-afzelechin, but lack of material has prevented further study.

It is noteworthy that whereas *Afzelia* sp. heartwood (King, Clark-Lewis, and Forbes 1955) and *E. calophylla* kino (Hillis 1952) both contain kaempferol and (+)-dihydrokaempferol, the former contains (−)-*epi*afzelechin and the latter (+)-afzelechin.

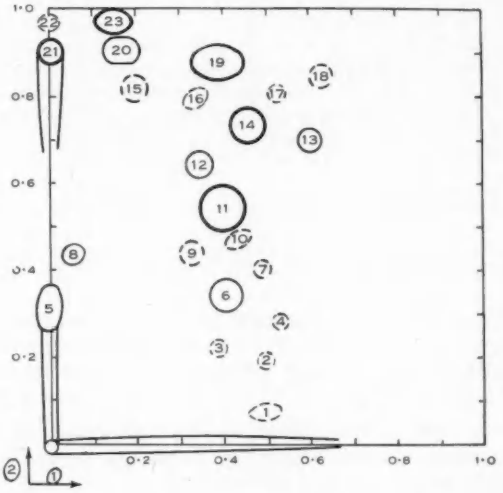


Fig. 1.—Paper chromatogram showing the major resolved components in the kino of *Eucalyptus calophylla*. The chromatogram was run first from left to right with 6 per cent. acetic acid and then upwards with *n*-butanol : acetic acid : water (6 : 1 : 2). The relative concentration of the components : – – weak ; — medium ; **—** strong. Components 2, 3, 4, 6, 7, 9, 11, 12, 14 gave a red colour with vanillin–hydrochloric acid. Components identified : 5, ellagic acid ; 6, gallocatechin ; 9, *epi*catechin ; 11, (+)-catechin ; 13, pyrogallol ; 14, (+)-afzelechin ; 19, aromadendrin ; 21, kaempferol.

Component 12 also gave an immediate red colour with vanillin–hydrochloric acid. Its chromatographic properties in "B.A.W." differ from those of (−)-*epi*catechin ($\Delta R_M = -0{\cdot}35$) by an amount similar to that between (−)-*epi*catechin and (−)-*epi*gallocatechin $\Delta R_M = +0{\cdot}38$). The relationship of component 12 to (+)-afzelechin ($\Delta R_M = 0{\cdot}22$) in 6 per cent. acetic acid is similar to that between (−)-*epi*catechin and (+)-catechin $\Delta R_M = 0{\cdot}23$) and (−)-*epi*gallocatechin and (+)-gallocatechin ($\Delta R_M = 0{\cdot}19$). These properties suggest that component 12 is *epi*afzelechin but it was found to be chromatographically different (Table 1) from the (−)-*epi*afzelechin isolated from *Afzelia* sp. (King, Clark-Lewis, and Forbes 1955). It is unlikely that the different $R_F$ values in

6 per cent. acetic acid can be due to the (−)- and (+)-forms of *epi*afzelechin as it has been found that the (−)-forms of the enantiomorphs of 7,3,4′-trihydroxyflavan-3,4-diol (Clark-Lewis and Roux 1959) and of gallocatechin (Roberts and Myers 1959), possess the same $R_F$ values as the (+)-form in "B.A.W." but lower $R_F$ values in aqueous solvents. It is noteworthy that of the six flavanols examined, only (−)-*epi*afzelechin departs significantly from the expected values. The amounts of pure component 12 obtained were insufficient for chemical investigation.

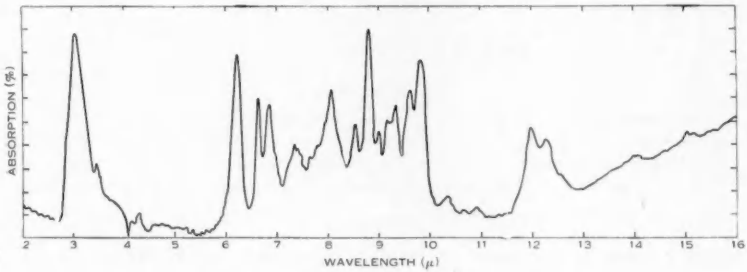


Fig. 2.—Infra-red spectrum of (+)-afzelechin (potassium chloride disk).

Pyrogallol (component 13) has also been isolated and identified. Other components present are being examined. Leucopelargonidin and leucocyanidin have been detected in the kino of *E. calophylla* (Hillis and Urbach 1958, 1959). Ganguly and Seshadri (1958) have indicated that they have isolated leucopelargonidin from this source. However, we have been able to detect only polymerized leucoanthocyanins in two-way chromatograms of our samples and have been unable to isolate any monomers.

TABLE 1

CHROMATOGRAPHIC PROPERTIES OF SEVERAL FLAVANOLS

| Compound | Component Number* | Chromatographic Properties | | | |
|---|---|---|---|---|---|
| | | In B.A.W. | | In 6% Acetic Acid | |
| | | $R_F$ | $R_M$† | $R_F$ | $R_M$† |
| (−)-*epi*Gallocatechin | — | 0·24 | +0·50 | 0·30 | +0·37 |
| (+)-Gallocatechin .. | 6 | 0·34 | +0·29 | 0·40 | +0·18 |
| (−)-*epi*Catechin .. | 9 | 0·43 | +0·12 | 0·29 | +0·39 |
| (+)-Catechin .. | 11 | 0·56 | −0·10 | 0·40 | +0·18 |
| | 12 | 0·63 | −0·23 | 0·35 | +0·27 |
| (−)-*epi*Afzelechin .. | — | 0·64 | −0·25 | 0·43 | +0·12 |
| (+)-Afzelechin .. | 14 | 0·74 | −0·45 | 0·47 | +0·05 |

* See Figure 1.

† $R_M = \log (1/R_F - 1)$; Bradfield and Bate-Smith (1950).

## II. Experimental

### (a) *Chromatographic Examination of* E. calophylla *Kino*

Methanol solutions of the samples were applied at a point 2 cm from both edges of the lower left-hand corner of Whatman No. 1 filter papers, 30·5 cm square, and chromatographed at 20 °C by the ascending method employing (i) 6 per cent. acetic acid, followed by (ii) *n*-butanol : acetic acid : water (6 : 1 : 2 v/v, B.A.W.) after drying in air for 4 hr. Both solvents travelled about 29 cm. The dried sheets were examined under ultraviolet light before and after fuming with ammonia (sp. gr. 0·88). then sprayed either with diazotized *p*-nitroaniline and sodium acetate (Swain 1952), vanillin–hydrochloric acid (Bate-Smith and Swain 1953) or ferric chloride freshly mixed with potassium ferricyanide (Kirby, Knowles, and White 1953). In almost all chromatograms the $R_F$ values of component 11 ((+)-catechin) were within $\pm R_F$ 0·05 of the values of 0·56/0·40 (B.A.W./6% acetic acid). The averages of the $R_F$ values of the different major components are shown in Figure 1.

Two samples of kino were collected from the interior of a recently felled log of *E. calophylla* and examined by the above techniques. One sample was in the form of a thick viscous liquid and by visual comparison the chromatogram of it appeared to contain more of the resolvable components than did that of the other sample which was semisolid.

On chromatographic examination the red ethyl ether extract from another sample of *E. calophylla* kino (Hillis 1952) contained most of the resolvable components in a purified form, and was used for further study.

All chromatographic comparisons of components were done by the technique of two-dimensional co-chromatography. A spot of the component was placed at the starting point on one paper sheet, and on another sheet was placed an equal amount of the component and in addition a spot of authentic material. Both sheets were developed at the same time by the above methods, the chromatograms examined and sprayed, and the resolved spot areas and colours compared. By this technique aromadendrin (component 19), kaempferol (21), ellagic acid (5) (Hillis 1952); catechin (11); *epi*catechin (9); gallocatechin (6) were identified.

Accurate $R_F$ values were determined by simultaneously chromatographing the flavanols in one dimension using either B.A.W. or 6% acetic acid. The results are given in Table 1.

### (b) *Primary Fractionation by Counter-Current Distribution*

The vacuum-dried ether extract (100 g) was dissolved in methanol (80 ml) at room temperature, ethyl ether (600 ml) added, and the whole vigorously shaken. The slightly turbid solution was added to the first five tubes of a Towers counter-current distribution apparatus (72 tubes, with a capacity of 150 ml of each phase). Each tube contained 150 ml of distilled water with the exception of the fifth which contained 70 ml. The mixture was fractionated with ethyl ether using a shaking time of 1·5 min (48 shakes) and settling time of 1·5 min. Samples of the contents of the 72 tubes and the 18 aliquots (A-R) discharged from the final tube were examined chromatographically using B.A.W. The contents of the tubes and the aliquots were grouped (see Table 2), and the groups then extracted with ethyl ether (8 times) and ethyl acetate (8 times) and the extracts evaporated and weighed (see Table 2).

### (c) *Secondary Fractionation with Cellulose Columns*

The cellulose columns were prepared by mixing thoroughly 250 g cellulose ("Solka Floc", Brown Co., Mass., U.S.A., purified by the method of Campbell, Work, and Mellanby 1951) with acetone (1500 ml) and quickly pouring into a chromatographic column (26 × 5·0 cm dia.) fitted with a sintered glass disk. Immediately after the cellulose suspension had been added, the tap at the bottom of the column was opened. Acetone was added to the column until the cellulose had settled to a height of 46 cm, when the supernatant acetone and suspended cellulose was carefully poured off. Distilled water (3 l.) was then passed through the column.

Samples of fraction 3 (7 g) (see Section II (*b*)) were dissolved in distilled water (17 ml) then cellulose powder (2·8 g) was added and well mixed. The thick mixture was gently poured onto the top of the above column with the aid of a pipette. The column was developed with distilled water, the eluate collected in volumes of 4 ml, and samples chromatographed in 6% acetic acid.

Tubes 1–18 contained component 13, tubes 19–52 components 13 and 14, tubes 53–90 component 14, tubes 91–134 components 14 and 12, tubes 135–215 component 12, tubes 216–400 component 12 and impurities. These different groups of tubes were extracted with ethyl acetate (10 times) and the extracts evaporated under vacuum.

A methanol extract of the column contained components 5 and 21.

TABLE 2

FRACTIONATION OF E. CALOPHYLLA KINO BY COUNTER-CURRENT DISTRIBUTION

| Fraction Number | Tube, or Aliquot Number | Components Present* | Weight of Fraction (g) |
|---|---|---|---|
| 1 | 1–16 | 1–11 | 6·3 |
| 2 | 17–38 | 8–12 | 20·0 |
| 3 | 39–72 R–H | 5, 11–17 | 6·0 |
| 4 | G–A | 15, 19–23 | 40·0 |
| 5 | Gum† | 5, 21 | 5·0 |

* For numbers see Figure 1.

† Gum coating tubes 1–6.

*(d) Examination of Components*

Melting points are uncorrected. Microanalyses were done by the C.S.I.R.O. Microanalytical Laboratory, Melbourne. A 5 cm capillary tube was used in the measurements of optical rotations.

(i) *Component 13* (*Pyrogallol*).—Long colourless needles formed in the concentrated extracts of tubes 1–18 (see Section II (*c*)), and the extracts was purified by vacuum sublimation. Altogether 1·3 g (0·1% of the kino) was obtained. The sublimate was identified as pyrogallol by co-chromatography, colour reactions, infra-red absorption spectrum, and m.p. and mixed m.p. 167–169 °C of the triacetate.

(ii) *Component 14* ((+)-*Afzelechin*).—Small colourless needles slowly formed in the brown concentrated extracts of tubes 53–90 (see Section II (*c*)). Recrystallization of the extracts from aqueous methanol (charcoal) yielded thin colourless needles (1·1 g; 0·1% of kino) which were readily soluble in water and organic solvents. A methanolic solution gave a faint green colour with aqueous ferric chloride, and a bright red colour with a solution of vanillin in concentrated hydrochloric acid (indicative of a flavan containing a phloroglucinol A ring). A methanolic solution did not become red in the magnesium– or zinc–hydrochloric acid test, or when treated with sodium amalgam and subsequently acidified. When gently warmed with concentrated sulphuric acid, afzelechin gave a red colour very similar to that given by catechin. A pale brown colour was obtained when the compound was heated at 100 °C with butanol–hydrochloric acid. The compound had m.p. 221–222 °C (decomp.), $[\alpha]_D^{20}$ +20·6° (*c*, 5% acetone : water (1 : 1)). Light absorption in methanol; $\lambda_{max.}$ 214, 277 mμ; $\lambda_{min.}$ 255 mμ. For infra-red spectrum see Figure 2 (Found: C, 65·7; H, 5·3%; mol. wt. (Rast), 275. Calc. for $C_{15}H_{14}O_5$: C, 65·7; H, 5·2%; mol. wt., 274).

(1) *5,7,4′-Trimethoxyflavan-3-ol.* Afzelechin (0·3 g) was dissolved in anhydrous ether (90 ml) and excess (4 times theoretical) diazomethane in ether was added. After 2 days the ether was rapidly washed with 4% NaOH, then water, and evaporated, when pale yellow needles formed which were twice crystallized from methanol : water (1 : 1). The long thin lustrous white needles (0·13 g) had m.p. 134–135 °C, $[\alpha]_D^{20}$ —0·9° (2% $C_2H_2Cl_4$) —5·7° (2% EtOH). Light absorption in methanol; $\lambda_{max.}$ 219, 274 mμ and $\lambda_{min.}$ 253 mμ (Found: C, 68·3; H, 6·6; $OCH_3$, 27·6%; mol. wt. (Rast), 319. Calc. for $C_{18}H_{20}O_5$: C, 68·3; H, 6·4; $3OCH_3$, 29·4%; mol. wt., 316). It was insoluble in warm 2N NaOH, and gave a red colour when warmed with concentrated sulphuric acid. The acetate, prepared with acetic anhydride and pyridine, crystallized from ethanol in colourless needles, m.p. 88–90 °C.

(2) *Potassium Permanganate Oxidation.* A solution of (+)-afzelechin trimethyl ether (0·04 g) in acetone (10 ml) was heated on a boiling water-bath and treated with powdered potassium permanganate (0·3 g) in small portions until a permanent pink colour was obtained. The insoluble material was separated by filtration, and suspended in 2N sulphuric acid (5 ml), treated with sulphur dioxide, and after boiling was filtered. After several days colourless needles formed in the filtrate. After washing with water, the crystals were placed between coverslips and heated on a microscope hot stage, m.p. 173–174 °C, unchanged on mixing with authentic *p*-anisic acid.

(3) *3,5,7,4′-Tetra-acetoxyflavan.* (+)-Afzelechin (0·03 g), acetic anhydride (1 ml), and pyridine (0·5 ml) were kept at 40 °C for 18 hr. The mixture was poured into crushed ice and the acetate recrystallized from aqueous acetic acid, the colourless needles melted at 109 °C (Found: C, 62·7; H, 5·2; Ac, 37·8%. Calc. for $C_{23}H_{22}O_9$: C, 62·4; H, 5·0; 4Ac, 38·9%). The acetate gave an immediate red colour with concentrated sulphuric acid.

(iii) *Component 12.*—The material in tubes 135–215 (Section II (*c*)) was readily soluble in water and organic solvents and contained some polymeric material. Some purification was achieved by a stepwise addition of light petroleum to an ethyl acetate solution. Further amounts were obtained by extracting the ether extracts of tubes 91–134 (Section II (*c*)) with neutral sodium borate solution and immediately acidifying the extract. All attempts to crystallize the material failed. Small amounts of chromatographically pure material were obtained by extracting bands of the material resolved on No. 3 Whatman paper. This material gave the colour reactions of a flavanol. Light absorption in methanol before or after the addition of sodium acetate or aluminium chloride was $\lambda_{max.}$ 222, 280 mμ; $\lambda_{min.}$ 253 mμ.

(iv) *Component 11* ((+)-*Catechin*).—Portion (2 g) of the pink granular material (fraction 2, see Section II (*b*)) was repeatedly recrystallized from water to give colourless needles (0·2 g) which were chromatographically pure and indistinguishable from (+)-catechin, m.p. and mixed m.p. 174–175 °C. Light absorption in methanol $\lambda_{max.}$ 214, 277 mμ, $[\alpha]_D^{20}$ +16·1° (acetone : water, 1 : 1) (Freudenberg 1933, +17°). Pentacetate, m.p. 131 °C; 5,7,3′,4′-tetramethyl ether, m.p. 141 °C; Freudenberg (1933) gives respectively 131–132 °C and 143–144 °C).

## III. Acknowledgments

The authors are indebted to Dr. J. W. Clark-Lewis, to Dr. H. G. C. King and to Dr. D. E. Hathway for samples of (−)-*epi*afzelechin, (−)-*epi*catechin and (−)-*epi*gallocatechin, and (+)-gallocatechin respectively, and to Mrs. M. Szilagyi and Miss E. Gloss for experimental assistance; to Mr. K. Harrington for infra-red spectra; and to the Chemical Research Laboratories, C.S.I.R.O., for the use of their Towers counter-current distribution apparatus.

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# DEGRADATION OF CARBOHYDRATES

## I. ISOLATION OF 3-DEOXYHEXOSONES

By E. F. L. J. ANET*

[*Manuscript received April* 19, 1960]

*Summary*

The two possible 3-deoxy-D-hexosones were prepared in high yields by the decomposition of diketoseamino acids in dilute aqueous solution at pH 5 and 100 °C. Small amounts of the corresponding epimeric aldoses were also isolated.

1,1′-(Carboxymethylamino)bis-[1-deoxy-D-fructose], the preparation of which has been improved, gave 3-deoxy-D-*erythro*hexosone which was characterized as the 2,4-dinitro- and 2,5-dichlorophenylosazones and triacetates, and as the 4-nitrophenylosazone. The 4-nitro- and 2,4-dinitrophenylosazones were identical with those prepared from 3-deoxy-D-*ribo*hexose. 1,1′-(Carboxymethylamino)bis-[1-deoxy-D-tagatose] yielded 3-deoxy-D-*threo*hexosone characterized as the 2,4-dinitrophenylosazone and triacetate and 2,5-dichlorophenylosazone.

Chromatographic evidence suggests that 3-deoxyhexosones are also formed during the decomposition of other substituted 1-amino-1-deoxyketoses.

A mechanism for the formation of 3-deoxyhexosones and of the epimeric aldoses from diketoseamino acids is proposed.

### I. INTRODUCTION†

The 3-deoxyhexosones have been postulated as intermediates in the degradations of sugars by alkali to metasaccharinic acids (Nef 1910), and by acids to furfurals (Wolfrom, Schuetz, and Cavalieri 1948). In spite of their theoretical importance they had not been isolated from any of these reactions nor had any been prepared. The two possible 3-deoxy-D-hexosones have now been prepared from the decomposition of diketoseamino acids, which are themselves intermediates (Anet 1959*b*) in the degradation of sugars by amino acids (Maillard reaction).

Diketoseamines were known to undergo rapid decomposition at about pH 5 to give a quantitative yield of the monoketoseamine, the other ketose moiety being converted to unknown carbonyl compounds (Anet 1959*a*). It has now been shown that, in the decomposition of two diketoseamino acids, one carbonyl compound, the 3-deoxyosone, predominates among the reaction products. The diketoseamino acids studied were: di-D-fructoseglycine (Ia) dihydrate, the only known crystalline diketoseamine, and di-D-tagatoseglycine‡ (Ib). Although highly unstable these diketoseamino acids were easily prepared

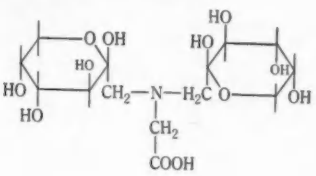
* Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

† A preliminary account has been given by Anet (1960*b*).

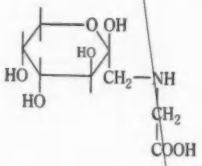
‡ 1,1′-(Carboxymethylamino)bis-[1-deoxy-D-fructose] and 1,1′-(carboxymethylamino)bis-[1-deoxy-D-tagatose] respectively.

in quantity in one step from D-glucose and D-galactose, respectively, and the sodium salt of glycine (Anet 1959*a*). A cleaner product was obtained by reducing the pH and the reaction time. The yield was then somewhat lower but larger quantities of the monoketoseglycine could be isolated.

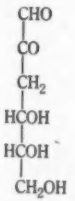
The diketoseglycines were decomposed by heating a dilute aqueous solution at about pH 5 for about 4 min at 100 °C. The unchanged compound and the monoketoseglycine (II) were removed with a cation-exchange resin. Over 90 per cent. of the neutral compounds then consisted of the corresponding 3-deoxy-D-hexosone (III). The impurities included 1–2 per cent. of each of the two corresponding epimeric aldohexoses, and less than 5 per cent. of a compound of unknown structure.



(Ia)



(IIa)



(IIIa)

(Open-chain form)

Ib, IIb, and IIIb have the opposite configuration at carbon four.

The crude 3-deoxy-D-hexosones were purified by chromatography on a paper column (see Table 2). The major portion of the deoxyosone could be obtained pure after one run, although the product showed multiple spots on paper chromatograms. The epimeric aldoses were also separated from each other, but each was contaminated with large amounts of deoxyosone. The D-mannose, D-glucose, and D-galactose were freed of deoxyosone by repeated column chromatography.

Pure 3-deoxy-D-*erythro*hexosone (IIIa) from Ia and 3-deoxy-D-*threo*hexosone (IIIb) from Ib both showed the same pattern on paper chromatograms. The main spot $R_{Glucose}$ 2·80–1·90 was elongated and trailed to two weak spots, $R_{Glucose}$ 1·36 and 1·06. When the main spot was eluted and rechromatographed the same pattern of three spots reappeared. The multiple spots indicate that the osones existed in at least three forms, possibly monomers and dimers which reached equilibrium slowly. The problem of the structure of the 3-deoxy-hexosones is similar to that of glucosone which also showed multiple spots on paper chromatograms (Bayne and Fewster 1956). Open-chain dicarbonyl forms, monocyclic hemiacetals, and the corresponding enols and hydrates (2,2-dihydroxy derivatives) are all possible. The absence of strong absorption in the ultraviolet however shows that the αβ-unsaturated aldehyde form is not present in large amounts. The mutarotation in water of the anhydrous 3-deoxy-hexosones could indicate the formation of a hydrate.

TABLE 1

SUBSTITUTED PHENYLHYDRAZONES OF 3-DEOXYHEXOSONES

| Osone | Substituent | Melting Point (°C decomp.) | $[\alpha]_D^{\prime}$ (constant) | Concentration* | Formula | Percentage Analyses | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Found | | | | Calculated | | | |
| | | | | | | C | H | N | Groups | C | H | N | Groups |
| D-*erythro*- | 2,4-Dinitro- | 266–267 | +800° | 0·5 | $C_{18}H_{18}O_{11}N_8$ | 41·7 | 3·7 | 21·4 | 0·0‡ | 41·4 | 3·5 | 21·5 | 0·0‡ |
| | Triacetate† | 184 | +588° | 0·24 | $C_{24}H_{24}O_{14}N_8$ | 44·6 | 3·9 | 17·1 | 20·2§ | 44·4 | 3·7 | 17·3 | 19·9§ |
| | 2,5-Dichloro- | 242 | +400° | 1·4 | $C_{18}H_{18}O_3N_4Cl_4$ | 45·2 | 4·0 | 11·3 | 29·9‖ | 45·0 | 3·8 | 11·7 | 29·6‖ |
| | Triacetate | 184 | — | — | $C_{24}H_{24}O_6N_4Cl_4$ | 47·8 | 4·1 | 9·4 | 20·9§ | 47·5 | 4·0 | 9·2 | 21·3§ |
| | 4-Nitro-† | 260–262 | +350° | 0·4 | $C_{18}H_{20}O_7N_6$ | 49·9 | 4·8 | 19·3 | — | 50·0 | 4·7 | 19·4 | — |
| D-*threo*- | 2,4-Dinitro- | 258–259 | −500° | 0·2 | $C_{18}H_{18}O_{11}N_8$ | 41·1 | 3·6 | 21·3 | 0·0‡ | 41·4 | 3·5 | 21·5 | 0·0‡ |
| | Triacetate | 209 | — | — | $C_{24}H_{24}O_{14}N_8$ | 44·3 | 3·8 | 17·6 | — | 44·4 | 3·7 | 17·3 | — |
| | 2,5-Dichloro- | 225 | −208° | 0·5 | $C_{18}H_{18}O_3N_4Cl_4$ | 45·1 | 3·9 | 11·3 | — | 45·0 | 3·8 | 11·7 | — |

* The solvent for the 2,4-dinitro- and 4-nitro-derivatives was pyridine–acetic acid 1 : 1, for the 2,5-dichloro-derivatives pyridine, and for the triacetate chloroform.

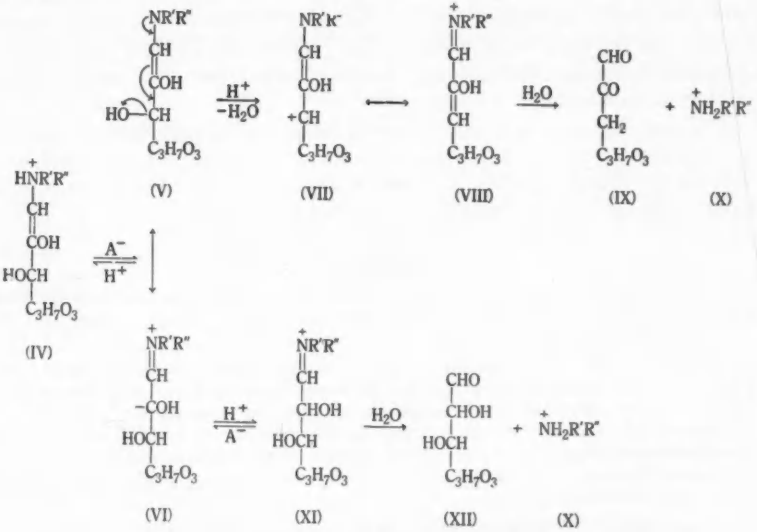
† Identical with the corresponding osazone from 3-deoxy-D-glucose.

‡ C—$CH_3$.

§ Acetyl.

‖ Cl.

Both 3-deoxy-D-hexosones readily gave crystalline substituted phenylosazones in the absence of acid catalysts. The analyses and properties of these derivatives and some of their triacetates are shown in Table 1. As criteria of identity of these osazones, the high melting points (with decomposition) were rather unsatisfactory but the very high optical rotations were very useful. The 2,4-dinitrophenylosazones were highly insoluble and were precipitated in over 90 per cent. yield in a pure crystalline form from each of the pure 3-deoxy-D-hexosones. The 4-nitro- and the 2,4-dinitrophenylosazones gave intense blue and violet colours respectively with alkali showing the presence of a 1,2-dicarbonyl group, which was probably in the 1,2-positions of the sugar. The 4-nitro- and




2,4-dinitrophenylosazones from IIIa were identical with those prepared from 3-deoxy-D-glucose (3-deoxy-D-*ribo*hexose), confirming the structure of IIIa. These two osazones have also been prepared previously from 3-deoxymannose (see Section II (*f*)) but the optical rotations were not recorded.

In the Lobry de Bruyn–Alberda van Ekenstein transformation of a ketose to the epimeric aldoses, formation of the deoxyosone is normally a side reaction. Both reactions are considered to proceed through a common intermediate, the 1,2-enediol of the sugar (Speck 1958). In the case of the diketoseamino acids the 3-deoxyhexosone was the main product and only traces of the epimeric aldoses were formed. Under the conditions used in the present work both reactions were irreversible and the products were so stable that the amounts of

the two classes of compounds were a measure of their rates of formation. The rapid rate of decomposition of the diketoseamino acids was probably due to their ready enolization, even in the absence of strong alkali or acid, to give the 1,2-enolammonium compound IV analogous to the 1,2-enediol from an aldose or ketose. Removal of a proton by basic catalysis would yield V⟷VI, analogues of the hybrid anions from an aldose (Speck 1958). Addition of a proton to V followed by dehydration would yield VII⟷VIII which would hydrolyse to the 3-deoxyosone (IX) and the amine (X). Alternatively, addition of a proton to VI would convert it to XI yielding the epimeric pair of aldoses (XIII) and the amine (X). The formation of VI from V involves a separation of charge and would therefore be less likely to occur than in the case of the hybrid anions of a 1,2-enediol from an aldose, where the corresponding change involves a movement of charge only. The proposed mechanism thus provides an explanation for the small amount of aldose formed during the decomposition of diketoseamino acids.

A 3-deoxyhexosone was also detected on paper chromatograms from the decomposition of l-deoxy-l-morpholino-D-fructose and of l-deoxy-l-toluidino-D-fructose in aqueous solutions adjusted to pH 4·5. It would thus appear that 3-deoxyhexosones can be formed from other ketoseamines.

## II. Experimental

Melting points are corrected. Solutions were evaporated under reduced pressure using rotary evaporators. The microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne.

(*a*) *Chromatography.*—Whatman No. 1 paper and *n*-butanol–acetic acid–water 4 : 1 : 1 were used for the paper chromatography. L.K.B. "Chromax" No. 4 columns were used for preparative paper chromatography. The eluates were contaminated with polyethylene from some of the column components. The fractions were concentrated and the residues were dissolved in a small volume of water, treated with a small quantity of charcoal, and filtered, removing the contaminants. Ion-exchange chromatography and paper electrophoresis were carried out as described previously (Anet 1959*a*).

(*b*) *Preparation of Di-*D*-fructoseglycine* (*cf. Anet 1959a*).—A solution of glycine (1 mole) and sodium hydroxide (0·75 mole) was evaporated to dryness. The residue was dried at 100 °C in a vacuum oven and ground to a fine powder. The glycine–sodium hydroxide powder (100 g) was rapidly added to a stirred solution of glucose (720 g) in water (80 ml) at 92 °C. The temperature rose from 92 to 95 °C after 7 min stirring. The mixture was then rapidly dissolved in degassed water (800 ml) and added to degassed ethanol (800 ml). Ten batches were combined and fractionated by displacement chromatography on a set of columns of sulphonated polystyrene resin (Partridge and Brimley 1952). The first column contained 8% cross-linked resin (20 lb), the other columns contained 4% cross-linked resin. The displacing agent was aqueous pyridine (0·5N ; 5 l.) followed by 0·5N ammonia. The effluent containing only difructoseglycine was bulked and concentrated to half volume and allowed to crystallize at +1 °C. The yield was 700 g of almost colourless plates of the dihydrate (14·5% yield from glycine ; 8% from glucose). Fructoseglycine (400 g, 15·5% from glycine) was also obtained as light brown prisms after one crystallization.

(*c*) *Decomposition of Difructoseglycine.*—Difructoseglycine dihydrate (22 g) was dissolved in 0·2N aqueous pyridine (120 ml) at 70 °C. The solution (pH 5·15) was poured into boiling water (800 ml) and heated, vigorously at first, for 4½ min, most of the time at the boiling point. The hot solution was poured onto crushed ice. The pH had risen to 5·8. An equal volume of degassed

ethanol was added to the cold solution and the mixture was passed through a column of sulphonated polystyrene resin to remove cations. The column was washed with 50% ethanol and the combined effluents were concentrated to a syrup, consisting mainly of the 3-deoxyosone (6·4 g dry weight).

The basic compounds on the column were displaced with 0·2N pyridine through a set of cation-exchange columns. Unchanged difructoseglycine emerged first, followed by fructose-glycine and a trace of an unknown compound of higher $R_F$ value and ionic mobility. The fractions containing difructoseglycine were concentrated to yield the dihydrate (4·5 g).

(i) *Isolation of 3-Deoxy-*D*-glucosone (3-Deoxy-*D*-erythro*hexosone*).* The crude 3-deoxyosone syrup was fractionated on a paper column using acetone–water 4 : 1 as eluant. The separation of the first run is shown in Table 2. Fractions 18–35 yielded 3-deoxyglucosone as a solid colourless froth on drying for 2 hr at 60 °C, $[\alpha]_D^{25}$ —2·5° (10 min)→1·5° (4 hr, constant) (*c*, 5·98 in water) (Found: C, 44·5; H, 6·6; O, 49·2%. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2; O, 49·4%). The compound showed no strong absorption in the ultraviolet region.

(ii) *Isolation of* D*-Mannose.* Fractions 36–46 (Table 2) were concentrated and re-run four times on the paper column, the D-mannose (70 mg) being finally separated from the osone and the glucose. The latter was added to fractions 47–57. The D-mannose with phenylhydrazine in ethanol, yielded the hydrazone, colourless needles, m.p. 192–195 °C (rapid heating, not depressed by an authentic specimen), $[\alpha]_D^{25}$ +30° (*c*, 0·53 in pyridine) (Found: N, 10·4%. Calc. for $C_{12}H_{18}N_2O_5$: N, 10·4%).

(iii) *Isolation of* D*-Glucose.* The D-glucose from fraction 36–46 above was added to fractions 47–57 (Table 2) and re-run on the paper column twice. The yield of D-glucose was 100 mg. It yielded the phenylosazone, m.p. 205 °C, not depressed by an authentic specimen.

(*d*) *Decomposition of Ditagatoseglycine.*—Ditagatoseglycine syrup (Anet 1959*a*) (6 g) was dissolved in warm 0·2N pyridine (30 ml) giving a pH of 5·0. The solution was poured into boiling water (200 ml) and heated for 4 min. The hot solution was cooled with ice and decationized as in Section II (*c*). The resulting solution contained mainly the 3-deoxyosone.

(i) *Isolation of 3-Deoxy-*D*-galactosone (3-Deoxy-*D*-threo*hexosone*).* The crude compound above was fractionated on a paper column using acetone–water 4 : 1 as eluant. The separation pattern was similar to that of 3-deoxy-D-glucosone in Table 2. Fractions 1–12 contained traces of compound U2 and 5-(hydroxymethyl)-2-furaldehyde. Fractions 14–20 and 29–41 gave the pure osone, but 21–28 contained traces of an aldose sugar, probably talose ($R_{\text{Glucose}}$ 1·4). Fractions 42–66 contained traces of D-galactose ($R_{\text{Galactose}}$ 1·00) and some osone. The 3-deoxy-D-*threo*-hexosone showed the same pattern on paper chromatograms as 3-deoxy-D-*erythro*hexosone, a major spot $R_{\text{Glucose}}$ 2·80–1·90, and two spots $R_{\text{Glucose}}$ 1·36 and 1·06 (Found: C, 44·2; H, 6·5%. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2%).

(ii) *Isolation of* D*-Galactose.* Fractions 42–66 were re-run on the paper column using ethanol–water 4 : 1 as eluant. The fractions containing D-galactose were converted to the phenylosazone, m.p. 186 °C, not depressed by an authentic specimen.

(*e*) *Substituted Phenylosazones from the 3-Deoxy-*D*-hexosones (see Table 1).*—The hydrazine (2 moles) and a 2% solution of the osone in 95% ethanol were refluxed for ½ hr with stirring. The crystalline osazone was filtered off from the hot reaction mixture and was washed with ethanol. The 2,5-dichlorophenylosazones (pale yellow needles) were recrystallized from ethanol. The 4-nitro- (orange prisms) and the 2,4-dinitrophenylosazones (orange needles), both insoluble in alcohol, were recrystallized from pyridine–acetic acid 1 : 1 in which solvent they gave a clear yellow-orange solution. In pyridine without acetic acid they gave a dark solution. When prepared from the chromatographically pure osones the osazones were pure and did not need recrystallization. The 2,4-dinitrophenylosazones were obtained in over 90% yield. The 4-nitro- and the 2,4-dinitrophenylosazones gave intense blue and purple colours respectively with alkali.

(i) The triacetates (see Table 1) were prepared by adding excess acetic anhydride to the solution used for the optical rotations of the parent compounds, and allowing the reaction mixtures to stand overnight. The reaction mixtures were taken up in chloroform, washed successively with water, dilute hydrochloric acid, and dilute sodium carbonate, and dried over sodium sulphate. The triacetates were recrystallized from chloroform–hexane.

(*f*) *4-Nitro- and 2,4-Dinitrophenylosazones from 3-Deoxy-*D*-glucose (3-Deoxy-*D*-ribo*hexose*).*—(i) A solution of 3-deoxy-β-D-glucose (Anet 1960*a*) (100 mg) and of 4-nitrophenylhydrazine (300 mg) in aqueous acetic acid (4 ml; 15%) was refluxed for 1 hr. The osazone was filtered, washed with aqueous acetic acid, water, and methanol–ether. Two recrystallizations from pyridine–acetic acid–water gave red prisms, m.p. 260–262 °C (decomp.), $[\alpha]_D^{25}$ +350° (*c*, 0·2 in pyridine–acetic acid 1 : 1) (Found: C, 49·9; H, 4·7; N, 19·3%. Calc. for $C_{16}H_{20}N_6O_7$: C, 50·0; H, 4·7; N, 19·4%). The 4-nitrophenylosazone of 3-deoxy-D-mannose has been reported as dark red prisms, m.p. 260–262 °C (decomp.) (Bolliger and Prins 1946).

TABLE 2

NON-BASIC PRODUCTS OF THE DECOMPOSITION OF DIFRUCTOSEGLYCINE

Fractionation on paper column with acetone–water 4 : 1

| Fraction | Constituent | $R_{Glucose}$* | Intensity of Spot*† |
|---|---|---|---|
| 1–9 | 5-(Hydroxymethyl)-2-furaldehyde‡ | 4·60 | Very weak |
| | Unknown U1 | 3·70 | Weak |
| 18–35 | 3-Deoxyglucosone | 2·80–1·90 | Intense |
| | | 1·36 | Weak |
| | | 1·06 | Very weak |
| 36–46 | 3-Deoxyglucosone | 2·80–1·90 | Medium to weak |
| | Mannose | 1·19 | Weak, in middle fractions only |
| | Glucose | 1·00 | Very weak, in last fraction only |
| 47–57 | Glucose | 1·00 | Medium |
| | 3-Deoxyglucosone | 2·80–1·90 | Weak |

* *n*-Butanol–acetic acid–water 4 : 1 : 1; spots revealed with alkaline silver nitrate.

† The 3-deoxyglucosone, all three spots, compound U1, and the 5-(hydroxymethyl)-2-furaldehyde all gave a strong pink colour with naphthoresorcinol.

‡ The 5-(hydroxymethyl)-2-furaldehyde was formed on the ion-exchange columns probably from U1.

(ii) The 2,4-dinitrophenylosazone was obtained in 96% yield after heating the reactants for 2 hr at 100 °C in 2N hydrochloric acid. Recrystallization from pyridine–acetic acid–water or ethyl acetate gave the osazone as orange needles, m.p. 265–267 °C (decomp.), $[\alpha]_D^{25}$ +794° (*c*, 0·5 in pyridine–acetic acid) (Found: C, 41·6; H, 3·6; N, 21·4%. Calc. for $C_{18}H_{18}O_{11}N_8$: C, 41·4; H, 3·5; N, 21·5%). The 2,4-dinitrophenylosazone of 3-deoxy-D-mannose has been reported as having m.p. 205 °C (decomp.) (Foster *et al.* 1953), but no optical rotation was given.

(*g*) *Detection of 3-Deoxyhexosones from Other* N*-Substituted 1-Amino-1-deoxyketoses.*—1-Deoxy-1-morpholino-D-fructose (0·2 g) in water (5 ml) was brought to pH 4·5 with acetic acid and heated at 100 °C for 5 min. The mixture was decationized with ion-exchange resin. The residue gave the characteristic spots on paper chromatograms ($R_{Glucose}$ 2·80–1·90, 1·35, and 1·05) of the 3-deoxyhexosones and gave an intense pink colour with naphthoresorcinol. A reductone (cf. Hodge and Rist 1953) was also present. 1-Deoxy-1-toluidino-D-fructose also gave the 3-deoxyhexosone spots but no reductone.

## III. ACKNOWLEDGMENTS

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# CHEMISTRY OF NON-ENZYMIC BROWNING

## XI. THE REACTIONS OF BISULPHITE WITH REDUCING SUGARS

By D. L. Ingles*

[*Manuscript received February* 19, 1960]

*Summary*

The reactions of reducing sugars with bisulphite have been studied at 100 °C and lower temperatures. Reducing sugars promoted the autoxidation of bisulphite to sulphur and sulphate, the ketoses being more effective than the aldoses. During the autoxidation the aldoses were oxidized, in part, to the corresponding aldonic acids. The ketose sugars reacted more rapidly and extensively than the aldoses, only 32 per cent. of unchanged fructose being recovered after 8 hr at 100 °C. Oxidation of the ketoses probably occurred via the osone and gave a complex mixture of keto acids. Ascorbic acid was converted to dehydroascorbic acid and an unidentified acid.

The reactions offer possible explanations for the formation of sulphate and the loss of bisulphite observed during storage of foods treated with sulphur dioxide. An oxidative mechanism has been suggested for the inhibition of non-enzymic browning by sulphur dioxide.

### I. Introduction

The oxidation of reducing sugars by aqueous bisulphite at 135 °C was reported by Hägglund, Johnson, and Urban (1930). However, the possible relevance of this reaction to the use of sulphur dioxide in foods was not emphasized by reviewers (Stadtman 1948; Gehman and Osman 1954; Joslyn and Braverman 1954) who considered that the relatively high temperatures used by Hägglund and co-workers produced basic structural changes in the sugars. Recently (Ingles 1959), this reaction was demonstrated at lower temperatures and its significance indicated for the inhibition of deteriorative changes in foods treated with sulphur dioxide. Some details of this oxidative reaction are discussed in the present paper.

### II. Autoxidation Products from Bisulphite

The reaction involves a promotion of the autoxidation of bisulphite by reducing sugars in the course of which part of the reducing sugar is oxidized. Thus when a reducing sugar is heated with bisulphite the main products of the reaction are sulphate, sulphur, and sugar acids. In Figures 1 and 2 are shown the rates of formation of sulphate and sulphur from the reaction at 100 °C of sodium bisulphite (4 moles) with glucose (1 mole) or fructose (1 mole) in the presence of water (25 g/100 g solids).

Fructose was more effective than glucose in promoting autoxidation of the bisulphite. Approximately 2 moles of sulphate was formed per g-atom of sulphur in agreement with Bertholet's equation for the autoxidation:

$$3H_2SO_3 \rightarrow 2H_2SO_4 + S + H_2O.$$

* Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

The yields of sulphate and sulphur per mole of reducing sugar increased with increasing concentration of bisulphite (see Figs. 3 and 4) until with 8 moles of bisulphite per mole of glucose (or 12 moles of bisulphite per mole of fructose)

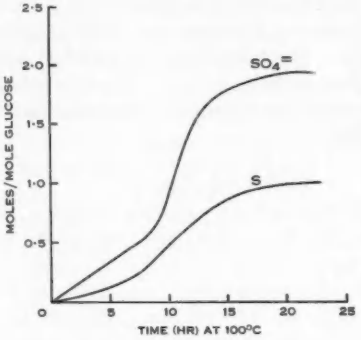


Fig. 1

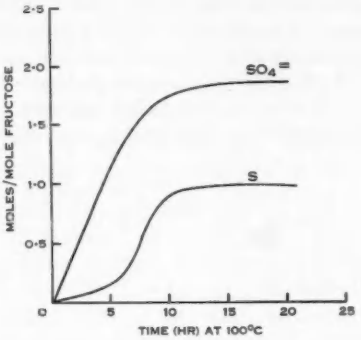


Fig. 2

Fig. 1.—Formation of sulphate and sulphur from the reaction of glucose (1 mole), sodium bisulphite (4 moles), and water (25 g/100 g solids).

Fig. 2.—Formation of sulphate and sulphur from the reaction of fructose (1 mole), sodium bisulphite (4 moles), and water (25 g/100 g solids).

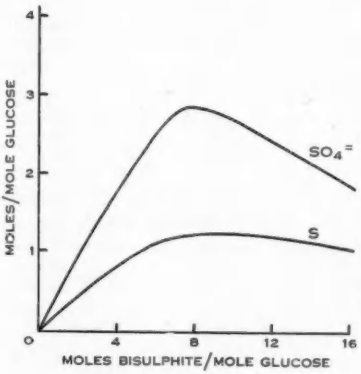


Fig. 3

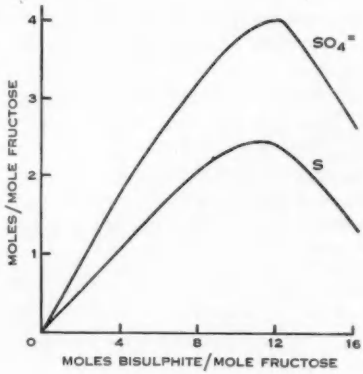


Fig. 4

Fig. 3.—Formation of sulphate and sulphur from the reaction of glucose (1 mole), sodium bisulphite, and water (25 g/100 g solids) at 100 °C for 20 hr.

Fig. 4.—Formation of sulphate and sulphur from the reaction of fructose (1 mole), sodium bisulphite, and water (25 g/100 g solids) at 100 °C for 8 hr.

the yields began to decrease slightly. With a ratio of 4 moles of bisulphite per mole of glucose there were formed 0·8 g-atom of sulphur and 1·8 moles of sulphate, but only 0·34 mole of glucose was converted to acids (see Table 1).

Experiments at 50, 37, and 25 °C showed that at these temperatures also both sulphate and sulphur were formed. Other reducing compounds, galactose, xylose, arabinose, lactose, sorbose, fructose, ascorbic acid, and 1-deoxy-1-glycinofructose also promoted the autoxidation of bisulphite. However, no reaction was observed after 72 hr at 100 °C with mannitol, sorbitol, α-methyl-D-glucoside, mannose, or glyoxal, or with bisulphite alone. Mannose and glyoxal formed very stable bisulphite addition compounds. These inhibited the reaction which appears to depend upon the presence of a free reducing group. The autoxidation was observed in both sealed and open tubes and the possibility of aerial oxidation of bisulphite to sulphate is thus eliminated.

### III. Reaction Products of Reducing Sugars

The reaction mixture, after filtration to remove precipitated sulphur, was treated with " Dowex 50 " resin ($H^+$form) and the acid effluent neutralized with barium hydroxide to remove sulphate and sulphite. The barium was next adsorbed on " Dowex 50 " ($H^+$form) and the released acids and lactones were adsorbed on " Dowex 1 " (carbonate form) (cf. Machell and Richards 1957). Neutral compounds and unreacted sugars were washed from the resin and estimated by weighing. The acids were desorbed from the " Dowex 1 " resin

Table 1

Yields of neutral and acid compounds from the reaction at 100 °C of reducing sugar (0·1 mole), sodium bisulphite (0·4 mole), and water (25 g/100 g solids)

| Compound Oxidized | Time (hr) at 100 °C | Neutral Fraction (%) | Crystalline Sugar Recovered* (%) | Acid† Fraction (%) |
|---|---|---|---|---|
| Glucose .. .. | 24 | 52·1 | 55·1 | 33·6 |
| Galactose .. .. | 24 | 44·5 | 60·0 | 43·0 |
| Xylose .. .. | 24 | 62·1 | 25·0 | 31·7 |
| Arabinose .. .. | 24 | 49·0 | 66·0 | 48·6 |
| Rhamnose .. .. | 24 | 54·0 | 77·5 | 39·8 |
| Lactose .. .. | 24 | 46·6 | 70·1 | 50·0 |
| Fructose .. .. | 8 | 32·1 | 0 | 56·0 |
| Sorbose .. .. | 8 | 22·1 | 10·0 | 59·0 |
| Ascorbic acid .. | 12 | 12·5 | 0 | 74·9 |

* As percentage of neutral fraction.

† Weighed as ammonium salt.

with ammonium carbonate solution which was evaporated to give the acid fraction. Removal of ammonium ions with " Dowex 50 " resin gave the free acids which were examined chromatographically. The quantitative results are shown in Table 1.

The neutral compounds consisted mainly of unchanged sugar much of which was recovered in crystalline condition in most cases (see Table 1). The neutral fractions from the aldose sugars reduced cold Fehling's solution but gave negligible precipitates with a cold aqueous perchloric acid solution of 2,4-dinitrophenyl-

hydrazine. The neutral fraction from the reaction of fructose contained 0·14 per cent. glucosone, which was isolated and identified as the 2,4-dinitrophenylosazone (cf. Ingles 1959). Under the conditions used to form this derivative neither fructose nor glucose forms any osazone.

The aldose sugars were oxidized to the corresponding aldonic acids (cf. Hägglund, Johnson, and Urban 1930; Menzinsky 1935) although other acids of low $R_F$ were invariably present. The nature of these acids is being investigated. Yields of acids ranging from 31·7 to 50·0 per cent. were obtained from reaction of the aldose sugars with bisulphite at 100 °C for 24 hr.

The ketose sugars and ascorbic acid reacted more rapidly and extensively than the aldoses. Only 32·1 per cent. of fructose remained after 8 hr at 100 °C. The reaction gave glucosone and a complex mixture of keto acids which included an ascorbic acid analogue. Fructose may be oxidized through glucosone to 2-ketogluconic acid which is known (Regna and Caldwell 1944) to rearrange in acid conditions to an ascorbic acid analogue. This latter compound will, like ascorbic acid (Table 1), oxidize readily. On heating fructose at 135 °C for long periods Hägglund, Johnson, and Urban (1930) isolated αβγ-trihydroxybutyric acid. Such an acid could result from extended oxidation of the intermediate acids now observed in the oxidation of fructose. Sorbose also gave a complex mixture of acids. Ascorbic acid formed dehydroascorbic acid and one other acid.

## IV. Reaction Intermediates

Hägglund, Johnson, and Urban (1930) suggested that sugar–bisulphite addition compounds were the reactive intermediates. However, the formation of such compounds appears to hinder the reaction in the case of the aldoses and to prevent it at 100 °C with mannose and glyoxal. Bisulphite addition compounds have not been observed with fructose (Braverman 1953; Frahn and Mills 1956) and the ketose sugars studied here reacted more rapidly than the aldoses (cf. Table 1). Hägglund, Johnson, and Urban (1930) found that fructose reacted more slowly than glucose. However, they measured the rate of oxidation by the decrease in titration with Fehling's solution. This procedure is invalid since the chief products of the oxidation of the ketoses also reduce Fehling's solution. Their results were therefore too low.

Kolthoff and Miller (1941) suggested that dithionite and thiosulphate were formed in the reduction of sulphite while dithionate and sulphate are the main products of the oxidation of sulphite (Foerster and Friessner 1902). The autoxidation of bisulphite may therefore involve both dithionite and dithionate as shown:

$$4H_2SO_3 \rightarrow H_2S_2O_4 + H_2S_2O_6 + 2H_2O.$$

Dithionate is known to decompose on heating to sulphate and sulphite. Dithionite was found to autoxidize when heated with water at 100 °C for 8 hr to form 0·79 mole of sulphate and 0·76 g-atom of sulphur per mole.

Oxidation of the reducing sugar may result from the action of one or more of the intermediates of the autoxidation: bisulphite, dithionate, and dithionite. This oxidation probably involves an initial abstraction of hydrogen by either an

ionic (Friedberg and Kaplan 1957) or free radical mechanism (Franck and Haber 1931; Backstrom 1934; Kharasch, May, and Mayo 1938).

Sorber (1944) observed the formation of sulphate in stored sulphured foods, although not in sufficient quantity to account for all the bisulphite lost. The promotion of the autoxidation of bisulphite by reducing sugars to sulphate and sulphur offers a possible explanation of these observations. For the inhibition of non-enzymic browning a new oxidative mechanism in addition to the three mechanisms considered by Stadtman (1948) has been proposed (Ingles 1959). Such oxidation reactions might serve to prevent aldol condensations (Hodge 1953) which would otherwise lead to the formation of brown pigments or polymers.

## V. Experimental

(*a*) *Estimation of Sulphate and Sulphur.*—Reducing sugar (0·01 mole), sodium bisulphite (0·04 mole), and water (25 g/100 g solids) were heated at 100, 50, 37, and 25 °C for different periods. The reactants were heated in open glass tubes at 100 °C and in sealed tubes at the lower temperatures. The pH of the reaction mixture, 4·2, remained essentially constant. Sealed- and open-tube experiments at 100 °C proceeded in the same way but the former occasionally exploded and were not therefore persisted with. After reaction the mixture was boiled for ½ hr with an excess of 0·5N hydrochloric acid to remove sulphite. Precipitated sulphur was then filtered off, dried, and extracted with carbon bisulphide. After evaporation of the carbon

Table 2

Yields of sulphate (moles) and sulphur (g-atoms) per mole of reducing sugar at 50, 37, and 25 °C

Reaction mixture: Reducing sugar (0·01 mole), sodium bisulphite (0·04 mole), and water (25 g/100 g solids)

| Compound Oxidized \ Time of Heating | 10 Weeks at 50 °C | | 16 Weeks at 37 °C | | 24 Weeks at 25 °C | |
|---|---|---|---|---|---|---|
| | Sulphur | Sulphate | Sulphur | Sulphate | Sulphur | Sulphate |
| Glucose .. .. | — | — | 0 | 0·10 | 0 | 0·11 |
| Fructose.. .. .. | 0·60 | 1·4 | 0·06 | 0·21 | 0 | 0·12 |
| Ascorbic acid .. .. | 0·80 | 1·3 | 0·75 | 1·4 | 0·09 | 0·54 |
| 1 - Deoxy - 1 - glycino-fructose .. .. | 0·21 | 0·62 | 0·08 | 0·29 | 0 | 0·12 |
| Sorbose .. .. | — | — | 0·07 | 0·34 | 0·03 | 0·27 |

bisulphide the residual sulphur was weighed. Sulphate was determined in the aqueous filtrate by precipitation with barium chloride. The results obtained at 100 °C are shown in Figures 1 and 2. The results for the lower temperatures (50, 37, and 25 °C) are shown in Table 2. Similar experiments were carried out at 100 °C with varying ratios of bisulphite to reducing sugar and the results are shown in Figures 3 and 4.

(*b*) *Examination of Reaction Products formed from Reducing Sugars.*—(i) *Chromatography and Sprays used.* Paper chromatograms were developed with butanol–acetic acid–water (4 : 1 : 1 v/v) and sprayed with silver nitrate and sodium hydroxide for carbohydrates, with ferric chloride–hydroxylamine for acid lactones, and with 2,4-dinitrophenylhydrazine–sodium hydroxide for carbonyl compounds.

(ii) *Separation of Acid and Neutral Fractions.* The reaction mixture obtained by heating reducing sugar (0·1 mole), sodium bisulphite (0·4 mole), and water (25 g/100 g solids) was diluted

with 500 ml water and filtered to remove precipitated sulphur. The filtrate was passed through a column of " Dowex 50 ", $H^+$form (200 g), and the acid effluent was neutralized with barium hydroxide to pH 10·0. Precipitated barium sulphate and sulphite were filtered off and the barium removed from the filtrate by retreatment with " Dowex 50 ", $H^+$form (200 g). The acid effluent was neutralized with " Dowex 1 ", carbonate form, and allowed to stand for 6 hr to ensure adsorption of lactones. The resin was washed to remove neutral compounds which were weighed after evaporation. Acids were desorbed from the " Dowex 1 " resin with saturated ammonium carbonate solution (1 l.). Evaporation gave the acid fraction which was weighed as the ammonium salt. The results of these experiments are shown in Table 1.

(iii) *Constituents of the Neutral Fractions.* Paper chromatography of the neutral fractions showed that in each case unchanged reducing sugar was the main component. On standing, the neutral compounds crystallized except in the case of fructose and ascorbic acid. The crystals were mixed with 50% ethanol, filtered, washed with 80% ethanol, dried, and weighed. The quantities of crystalline sugar recovered are given in Table 1. Treatment of the neutral fraction from fructose (0·36 g) with a 4% solution of 2,4-dinitrophenylhydrazine (50 ml) in aqueous perchloric acid (50%) gave glucose 2,4-dinitrophenylosazone (0·016 g) after 1 hr at 25 °C (cf. Ingles 1959). Similarly the sorbose neutral fraction gave 0·015 g of osazone under the same conditions. The neutral fractions from the aldose sugars however produced only a slight cloudiness with 2,4-dinitrophenylhydrazine and gave no measurable precipitate.

TABLE 3

IDENTITY OF ACIDS PRESENT

| Sugar Oxidized | $R_{Glucose}$ Values of Acid Constituents | $R_{Glucose}$ Value of Identified Constituent | | |
|---|---|---|---|---|
| | | Acid | | Lactone |
| D-Glucose .. | 0·26, 1·0, 2·53 | Gluconic .. | 1·0 | 2·53 |
| D-Galactose .. | 0·25, 0·90, 1·98 | Galactonic .. | 0·90 | 1·98 |
| D-Xylose .. .. | 0·25, 1·64, 2·97 | Xylonic .. .. | 1·64 | 2·97 |
| L-Arabinose .. | 0·32, 1·20 | Arabonic .. .. | 1·20 | — |
| L-Rhamnose .. | 0·18, 2·54, 3·6 | Rhamnonic .. | 2·54 | 3·6 |
| Lactose .. .. | 0·10 | | | |

(iv) *Constituents of the Acid Fractions.* (1) *Acids from Glucose:* The acid fraction from glucose (7·2 g) as the ammonium salt, was dissolved in water (50 ml) and passed through a column of " Dowex 50 ", $H^+$form (50 g). The acid effluent was neutralized with " Dowex 1 ", carbonate form (50 g), and the resin and adsorbed acids treated with 4N acetic acid (100 ml) and transferred to a column. The adsorbed acids were then displaced with 0·1N hydrochloric acid and the collected fractions (20 ml each) were examined by paper chromatography. Fractions 1–10 contained gluconic acid (4·6 g) isolated as the calcium salt, $[\alpha]_D^{25}$ +8·2° (c, 2% in water). Hudson and Isbell (1929) find $[\alpha]_D^{25}$ +8·5° (c, 3% in water) (Found: C, 33·4; H, 5·5; Ca, 9·2%. Calc. for $(C_6H_{12}O_7)_2Ca$: C, 33·4; H, 5·1; Ca, 9·3%). Fractions 11–18 contained an acid, $R_{Glucose}$ 0·26, which has not yet been identified.

(2) *Acids from Glucose and Other Aldoses:* The acid fractions from the aldose sugars were treated with " Dowex 50 ", $H^+$form, to yield the free acids which were examined by paper chromatography in butanol–acetic acid–water. The identity of the acids present was established by comparison with the $R_{Glucose}$ values of authentic acid samples run in the same solvent. The results are presented in Table 3.

The lactose acid, $R_{Glucose}$ 0·10, gave gluconic acid on acid hydrolysis and is presumed to be lactobionic acid.

(3) *Acids derived from Fructose, Sorbose, and Ascorbic Acid :* The acid fraction from fructose was treated as described (see (iv) (1) above) for the glucose acids. Displacement chromatography showed the acids given in Table 4.

The compound, $R_{Glucose}$ 2·3, in fraction 6 was a reductone which reduced cold silver nitrate. All the acids showed carbonyl activity when sprayed with an ethanolic solution of 2,4-dinitrophenylhydrazine followed by sodium hydroxide solution.

TABLE 4

ACIDS OBTAINED BY DISPLACEMENT CHROMATOGRAPHY

| Fraction number .. | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|
| $R_{Glucose}$ value of constituents | 1·1<br>2·3 | 0·33<br>1·1 | 0·33<br>1·1<br>1·4 | 0·33<br>0·6 | 0·6 |

The acids derived from sorbose had $R_{Glucose}$ values 0·33, 1·1, and 0·6 and all gave positive carbonyl reactions. From ascorbic acid there were obtained two acids $R_{Glucose}$ 1·4 and 2·4. The compound of higher $R_{Glucose}$ value was chromatographically identical with authentic dehydroascorbic acid.

(*c*) *Reaction of Bisulphite with Sorbitol, Mannitol, Glyoxal, and α-Methyl-D-glucoside.*—No reaction was observed when these compounds were heated at 100 °C for periods of 72 hr with sodium bisulphite and water.

(*d*) *Autoxidation of Sodium Dithionite.*—Sodium dithionite (1·9 g) and water (25 g/100 g solids) were heated at 100 °C for 8 hr. Sulphate and sulphur were estimated as described in (*a*) above. The yields obtained were : sulphur 0·76 g-atom/mole ; sulphate 0·79 mole/mole.

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# STUDIES IN THE NATURAL COATING OF APPLES

## V. UNSATURATED AND MINOR SATURATED ACIDS OF THE CUTICLE OIL

By J. B. DAVENPORT*

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*Summary*

Fatty acids soluble in acetone at —18 °C comprised 48·5 per cent. of the cuticle oil of ripe Granny Smith apples. This fraction had the following percentage composition: oleic acid 28, linoleic 49, linolenic 5, palmitic 1, and myristic 1. Lauric acid was also identified, and evidence for the presence of small amounts of caprylic, enanthic, and caproic acids was obtained by gas chromatography.

### I. INTRODUCTION

Parts I and II of this series (Huelin and Gallop 1951*a*, 1951*b*) described four fractions—oil, wax, ursolic acid, and " cutin "—occurring in the natural coating of Granny Smith apples, and discussed changes in these fractions during storage. Work has continued on the chemical composition of the oil, and Part III of this series (Davenport 1956) described the identification of stearic and arachidic acids as the major components of the saturated acid fraction. The present paper describes the identification of the unsaturated acids, which are the major acid components, and lower saturated acids.

After the saturated acid fraction was crystallized from acetone at —18 °C (Davenport 1956) the acids from the mother liquor were converted to their methyl esters and separated by fractional distillation (Table 1). The methyl esters of the $C_{18}$ acids were further fractionated by urea adduction (Table 2), and converted to hydroxamic acids which were separated by partition chromatography (Davenport 1955). The hydroxamic acids were crystallized and oleic, linoleic, and linolenic acids were identified. Palmitic, myristic, and lauric acids were identified as their hydroxamic acids, and evidence was obtained by gas chromatography for the presence of caproic, enanthic, caprylic, lauric, and myristic acids in trap material from the distillation. Oleic, linoleic, linolenic, palmitic, and myristic acids comprised 28, 49, 5, 1, and 1 per cent. of the unsaturated fraction respectively.

Halogen-containing substances detected in the trap material were probably derived from spray residues; they were not detected in oil from apples not sprayed with DDT. Photochemical decomposition of DDT (Wichmann *et al.* 1946) on the apple releases chlorine compounds which may combine with unsaturated material in the cuticle oil.

* Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

From the present data and those of Part III (Davenport 1956) the approximate percentage composition of the fatty acids of the oil is as follows: oleic 22, linoleic 39, linolenic 4, palmitic 1, stearic 7, arachidic 6, behenic 1, and myristic and lauric less than 1. The unsaturated acids show the composition

TABLE 1

DISTILLATION DATA

| Fraction | Weight (g) | Boiling Point (°C/1·0 mm) | Specific Gravity (25 °C/4 °C) | $n_D^{25}$ |
|---|---|---|---|---|
| 1 | 0·9 | 108–116 | | 1·4598 |
| 2 | 2·7 | 116–147 | 0·8928 | 1·4552 |
| 3 | 2·6 | 147–150 | 0·8790 | 1·4539 |
| 4 | 2·6 | 152 | 0·8791 | 1·4561 |
| 5 | 3·3 | 152 | 0·8797 | 1·4570 |
| 6 | 55·5 | 152 | 0·8791 | 1·4570 |
| 7 | 2·6 | 152 | 0·8790 | 1·4568 |
| 8 | 2·6 | 152–153 | 0·8796 | 1·4569 |
| 9 | 2·6 | 153–156 | 0·8802 | 1·4571 |
| 10 | 2·2 | 156–167 | 0·8845 | 1·4585 |
| 11 | 2·4 | 167–191 | 0·9023 | 1·4606 |

typical (Hilditch 1947) of seed fats of Rosaceae, the family to which the apple belongs. The high proportion of stearic and arachidic acids in the saturated fraction is unusual among vegetable oils, where palmitic acid usually pre-

TABLE 2

DATA FOR UREA ADDUCTS FROM FRACTION 6

| Fraction | Temperature of Adduction | Weight of Adduct (g) | Weight of Isolated Esters (g) | Specific Gravity (25 °C/4 °C) | $n_D^{25}$ | Iodine Value | Diene (%) | Triene (%) | Calc. Iodine Value* |
|---|---|---|---|---|---|---|---|---|---|
| 6A | Room | 27·1 | 6·8 | 0·8719 | 1·4519 | 99·5 | 17·7 | 1·5 | 100·0 |
| 6B | Room | 95·0 | 23·1 | 0·8768 | 1·4560 | 136·4 | 50·7 | 5·5 | 137·3 |
| 6C | 1 °C | 57·0 | 14·6 | 0·8832 | 1·4604 | 174·8 | 91·8 | 8·2 | 179·3 |
| 6D | —10 °C | 15·0 | 4·0 | 0·8856 | 1·4613 | 175·8 | 77·2 | 11·4 | 162·8 |
| 6E | Not adducted | — | 0·9 | — | 1·4722 | — | 23·0 | 14·0 | — |

* Calculated from per cent. diene and per cent. triene, assuming in the case of A and B that the remainder of the material was methyl oleate.

dominates. Thompson (1951) identified saturated acids from $C_1$ to $C_6$ in the volatile products of Granny Smith apples, and Chibnall *et al.* (1931) showed that $C_{28}$ and higher acids probably occur in the cuticle wax of apples. An intermediate series of even-numbered acids from $C_{12}$ to $C_{22}$ occurs in the cuticle

oil. This wide range of chain length of saturated acids and the virtual absence of any unsaturated acids of chain-length other than $C_{18}$ suggest that the two classes of fatty acids are formed by separate biosynthetic pathways.

## II. Experimental

The acid and iodine values were determined by the Official and Tentative Methods of the American Oil Chemists' Society (1946, 2nd Ed.). The hydroxyl values were determined by the semimicro procedure of Ogg, Porter, and Willits (1945). All melting points, unless stated to be micro, were determined in a Herschberg apparatus using calibrated total immersion thermometers. The micromelting points were determined on a hot stage using direct microscopic observation. Gas chromatography was carried out with a 1 m × 4 mm copper tube packed with a mixture of adipate polyester of diethylene glycol (1 g) (Lipsky and Landowne 1958) and "Celite 545" (4 g, 60–80 mesh), and a hot-wire detector. Nitrogen was used as the carrier gas.

Alkaline isomerization was carried out with 1·0M potassium *tert.*-butoxide for 2 hr at 100 °C (Davenport, Birch, and Ryan 1956), the percentages of the various fatty acids being calculated from the following equations:

$$\% \text{ triene acid} = 1{\cdot}314k_{268},$$
$$\% \text{ diene acid} = 1{\cdot}013k_{233} - 0{\cdot}734k_{268},$$

where $k$ is $E^{0\cdot1\%}_{1\text{ cm}}$.

The isolation of the saturated fatty acids from a sample of oil derived from 40 bus. of apples was described in Part III of this series (Davenport 1956). These acids separated on cooling a 10% solution of the total acids in acetone to —18 °C. The mother liquor yielded on evaporation 97·0 g of acids, predominantly unsaturated, which comprised 48·5% of the oil and had the following characteristics: acid value 199, iodine value 177·1, ultraviolet absorption $E^{1\%}_{1\text{ cm}}$ 22 at 232 mμ (max.) and $E^{1\%}_{1\text{ cm}}$ 13 at 268 mμ (max.). The acids were esterified with diazomethane and the methyl esters had a hydroxyl value of 21·5 and $[\alpha]^{20}_{D}$ +0·7±0·2 (acetone). The methyl esters were then distilled in a spinning-band fractionating column (Murray 1951) with dioctyl sebacate as a chaser. The distillation data are listed in Table 1.

*Fraction 2.*—This fraction was converted to hydroxamic acids by treatment with an alkaline solution of hydroxylamine in methanol at room temperature. The isolated hydroxamic acids were separated into a solid, insoluble in light petroleum, and an oil soluble in light petroleum. The solid fraction was separated by partition chromatography on a cellulose column (Davenport 1955) into two bands of peak effluent volumes 1125 and 1600 ml respectively. The first band yielded crystals, m.p. 102·8–103·8 °C, undepressed by admixture with palmitohydroxamic acid and the second crystals, m.p. 97·9–98·4 °C, undepressed on admixture with authentic myristohydroxamic acid.

The liquid fraction on chromatography gave two bands of peak effluent volumes 900 and 1225 ml respectively. These on evaporation and crystallization from light petroleum at 0 °C yielded crystals, m.p. 60·7–61·6 °C (micro), and 38·5–39·5 °C (micro), respectively. The former did not depress the m.p. of oleohydroxamic acid and the latter the m.p. of linoleohydroxamic acid.

*Fraction 6.*—This fraction and 50 g urea were dissolved in 350 ml of methanol with heating and allowed to cool slowly to room temperature, and the adduct filtered off. Another 120 g of urea and 150 ml of methanol were added to the mother liquor, which was again heated and allowed to cool to room temperature. A further batch of adduct was filtered off. On cooling the mother liquors to 1 °C a third batch of adduct separated. After filtering, 25 g of urea was dissolved in the mother liquor, which was cooled to —10 °C, yielding a final batch of adduct. The methyl esters were recovered from the various adducts and the final mother liquors by dilution with water and extraction with diethyl ether. The methyl esters were analysed by iodine values and alkaline isomerization. The yields and analyses are summarized in Table 2. Fraction 6E, from its smell, analytical values, and refractive index, apparently contained a high proportion of auto-oxidation products.

*Fraction 6A.*—A sample was converted to the hydroxamic acid, and crystallized from light petroleum and methanol to final m.p. 62·0–62·8 °C, undepressed on admixture with authentic oleohydroxamic acid. This material was oxidized by the procedure of Lemieux and von Rudloff (1955) yielding a dicarboxylic acid which after crystallization from benzene and water melted at 102·7–104·0 °C (micro), undepressed by admixture with azelaic acid.

*Fractions 6B and 6C.*—These fractions were combined. A sample was hydrogenated yielding methyl stearate, m.p. 38·2–38·9 °C (micro). Saponification yielded stearic acid, m.p. 69·7–70·5 °C, undepressed on mixing with an authentic sample.

*Fraction 6D.*—Conversion to the hydroxamic acids and partition chromatography gave two bands of peak effluent volumes 1175 and 1675 ml respectively. The first band yielded crystals which after recrystallization from light petroleum melted at 36·5–37·3 °C (micro), undepressed by admixture with linoleohydroxamic acid. The second band likewise gave linolenohydroxamic acid, m.p. 30·0–30·8 °C, undepressed by admixture with an authentic sample.

*Distillation Trap Material.*—About 5 ml of material which collected in the liquid air trap during the distillation was redistilled through a Podbielniak microspinning-band fractionating column. It gave four fractions as follows:

| Fraction | B.P. (°C/50 mm) | Volume (ml) | $n_D^{25}$ |
|---|---|---|---|
| A | 103–108 | 0·2 | 1·4928 |
| B | 108–121 | 0·3 | 1·4997 |
| C | 121–122 | 0·5 | 1·5076 |
| D | 122–147 | 0·6 | 1·4955 |

The final trap material gave a positive Beilstein test for halogen. B, although of the same boiling point as methyl laurate (b.p./50 mm 121 °C, $n_D^{25}$ 1·4298), had a much higher refractive index and hence was treated with urea, but yielded only 11·3 mg of adductable material. This was converted to the hydroxamic acid, which after crystallization from light petroleum melted at 92·1–92·9 °C (micro). Laurohydroxamic acid has m.p. 94 °C (Inoue and Yukawa 1940). The non-adductable material gave a single peak in the gas chromatogram (retention time 31 min, 172 °C, nitrogen flow rate 44·2 ml/min), and had an infra-red spectrum identical with that of dimethyl phthalate which had been used as a manometer liquid in the large spinning band column. Apparently in the distillation at 1·0 mm some of this material had collected in the trap. This explains the high refractive indices of the distillation fractions.

Gas chromatography of D gave seven peaks (retention times 3·5, 5·0, 6·7, 10·5, 12·5, 16·3, and 23·3 min, 171 °C, nitrogen flow rate 49·2 ml/min). The third peak is probably methyl laurate and the fifth peak is probably methyl myristate. Methyl palmitate under these conditions has a retention time of 29 min and hence the material appears to be a complex mixture of methyl esters from acids between $C_{10}$ and $C_{16}$. However, some peaks may represent not methyl esters but auto-oxidation products of unsaturated esters. Gas chromatography of the final trap material gave eight peaks (retention times 0·5, 1·0, 2·5, 3·7, 7·7, 12·7, 16, and 37 min, 96 °C, nitrogen flow rate 50·8 ml/min). The fourth, fifth, and sixth peaks corresponded to the peaks given by methyl caproate, methyl enanthate, and methyl caprylate respectively. Some of the other peaks are probably associated with the halogen-containing material. Oil from apples harvested in 1956 which had not been sprayed with DDT contained no volatile halogen-containing compounds.

The percentage composition of fraction 6 (Tables 1 and 2) was estimated from the iodine values, extinction values after isomerization, and values for specific gravity and refractive index. The composition of the other fractions in Table 1 was estimated from the boiling points and values for specific gravity and refractive index. From these results the approximate composition of the total unsaturated acid fraction was obtained.

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# AMINO ACIDS AND PEPTIDES

## VI. STUDIES ON CYSTINE AND αα′-DIMETHYLCYSTINE IN RELATION TO THE ALKALINE DEGRADATION OF PROTEIN DISULPHIDES*

By I. W. STAPLETON† and J. M. SWAN†

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*Summary*

The synthesis and properties of α-methyl-DL-cysteine and of racemic αα′-dimethylcystine and its dimethyl ester are recorded. The new disulphide is very much more stable to aqueous alkali than is cystine, its dimethyl ester is quite stable to methanolic triethylamine whereas cystine dimethyl ester breaks down readily with liberation of sulphur. These results support the β-elimination hypothesis for the alkaline degradation of cystine and its derivatives, namely, that attack occurs at the amino acid α-carbon atom, with synchronous displacement of a sulphenyl thiol anion, $RSS^-$.

### I. INTRODUCTION

The hydrolytic breakdown of cystine residues in proteins is a complex reaction. In strong alkali, cysteine residues appear which may then undergo further degradation, possibly to α-aminoacrylic acid residues and other products, while in dilute alkali the cystine is converted partly or even largely to combined lanthionine (for references see Blackburn and Lee 1956; Schöberl and Wagner

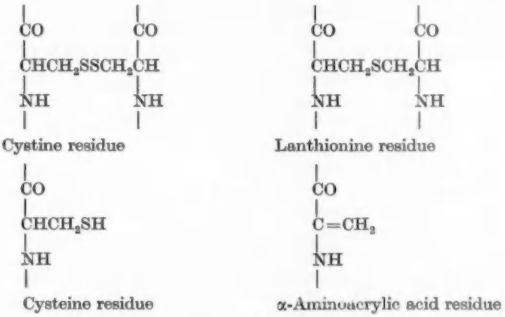



Fig. 1.—Representative structural formulae.

1956; Swan 1956; Cecil and McPhee 1959). Lanthionine residues may in turn undergo further decomposition (Zahn *et al.* 1957; Zahn and Kessler 1958). The fate of the sulphur lost from the protein is obscure, but hydrogen sulphide and sulphur (polysulphides) are commonly reported. Representative structural formulae are shown in Figure 1.

* For Part V of this series see Maclaren, J. A. (1958).—*Aust. J. Chem.* **11**: 360.

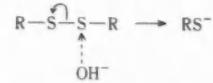
† Division of Protein Chemistry, C.S.I.R.O. Wool Research Laboratories, Melbourne.

Cystine and cysteine and their simple derivatives likewise undergo decomposition in alkali (see Tarbell and Harnish 1951 ; Schöberl and Wagner 1956 ; Swan 1956 ; Dann, Oliver, and Gates 1957 ; Cecil and McPhee 1959 ; and references quoted therein). Here again decomposition leads to the appearance of sulphur, sulphides (or organic thiols in the case of *S*-substituted cysteines), thiosulphate, sulphite, ammonia, carbonate, and various organic products including alanine, oxalic acid, pyruvic acid, 2-methylthiazolidine-2,4-dicarboxylic acid, and lanthionine.

A number of different mechanisms has been suggested for the breakdown of this class of disulphide in alkali. Schöberl (1933, 1936, 1941), Schöberl and Tausent (1956), and Speakman (1933, 1948) and Speakman and Stoves (1936) have supported Marriott's (1928) view that the primary action is one of direct hydrolysis of the disulphide bond, yielding thiol and sulphenic acid

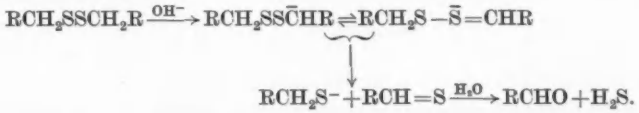
$$RSSR + H_2O \longrightarrow RSH + RSOH,$$

or in modern terms, displacement of thiol anion from the second sulphur atom by the nucleophilic hydroxyl ion (for review see Parker and Kharasch 1959) :



This theory has been widely quoted by protein chemists (Freudenberg and Wegmann 1935 ; Elsworth and Phillips 1938 ; Barbu, Lessian, and Macheboeuf 1949 ; Cecil 1950, 1951 ; Wieland 1954 ; Klotz *et al.* 1955 ; Wieland and Schwan 1956).

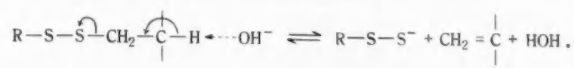
Rosenthal and Oster (1954) have proposed that for primary and secondary alkyl disulphides, the initial step in alkaline decomposition is removal of a proton from carbon *alpha* to one of the sulphur atoms. The carbanion formed then undergoes fission at the sulphur–sulphur bond by a nucleophilic elimination, giving thiol plus thioaldehyde ; the latter may then be hydrolysed to hydrogen sulphide and an aldehyde :



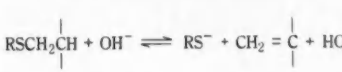
Recent observations on the alkaline decomposition of " dithiodiglycollic acid " (biscarboxymethyl disulphide ; Danehy and Krewz 1959) are fully in accord with the above mechanism. However, its relevance to protein disulphide groups is less certain. Danehy and Krewz state that the decomposition of bis-2-carboxyethyl disulphide in alkali follows a quite different pathway ; in this compound, as in cystine, the sulphur group is *two* carbon atoms distant from the carboxyl group. Moreover, on the Rosenthal and Oster mechanism it would be difficult to explain the formation of lanthionine and the non-appearance of aldehyde groups in alkali-treated wool (Cuthbertson and Phillips 1945 ;

Blackburn and Lee 1956) and also the great increase in sulphur lability which occurs when cystine or cysteine residues are incorporated into a peptide or protein (for references see Tarbell and Harnish 1951). Elliott, Asquith, and Hobson (1959) believe that small amounts of cysteine can play an important role in the alkaline decomposition of cystine, and that one stage in the reaction is the reduction of cystine to cysteine by sulphide ion formed during decomposition of the cysteine initially present. Dann, Oliver, and Gates (1957) have stressed earlier that the decomposition of cystine residues in a protein at an advanced stage of the reaction must be affected by the sulphide ions accumulating in solution.

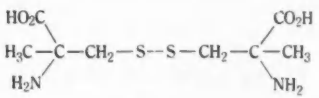
The work described in this paper was done in order to test the hypothesis (for reviews see Neuberger 1948; Tarbell and Harnish 1951; Parker and Kharasch 1959; Cecil and McPhee 1959) that the primary action of alkali on disulphides of the cystine type is nucleophilic attack by the $OH^-$ ion on a carbon atom *beta* to one of the sulphur atoms. This attack leads to ionization of the β-hydrogen (the so-called α-hydrogen atom of the amino acid) with synchronous elimination of a hydrodisulphide anion ($RSS^-$):



An analogous β-elimination could clearly be responsible for the decomposition of cysteine and *S*-substituted cysteines, including lanthionine:



On the β-elimination hypothesis, the compound αα′-dimethylcystine (I) would be expected to be much more stable than cystine, since the hydrogen atom essential for ionization is replaced by a methyl group:



(I)

The synthesis of this amino acid was therefore undertaken, so that its properties could be compared with those of cystine. A preliminary report (Swan 1957) of some of these results has appeared; since then, others have also recorded analogous syntheses of αα′-dimethylcystine (see Section III).

## II. Results and Discussion

α-Methyl-DL-cysteine was obtained by sodium–liquid ammonia reduction of *S*-benzyl-α-methyl-DL-cysteine, and was converted into the corresponding disulphide by aerial oxidation. The colour yield per mole of αα′-dimethylcystine in the phosphotungstic acid colorimetric analysis was found to be virtually

identical with that given by cystine, but no colour was obtained when Vassel's modification of the Fleming test for cystine (Block and Bolling 1951, p. 206) was applied. αα′-Dimethylcystine gave a red colour in the Sullivan test for cystine (Block and Bolling 1951, p. 198) but whereas the colour from cystine is stable to dithionite, that obtained with the methyl compound was found to be bleached rapidly.

The polarographic behaviour of αα′-dimethylcystine at various pH values both in the presence and absence of sulphite was found to be very similar to the behaviour of cystine (Kolthoff and Barnum 1941 ; Stricks and Kolthoff 1951). In particular, the new disulphide and also α-methylcysteine could be estimated in sulphite solution at pH 9–10 by amperometric titration with mercuric chloride, following the procedures developed for cystine and cysteine by Leach (1960). Both cystine (Brdicka 1933) and homocystine (Stern and Beach 1940) give rise to identical catalytic cobalt waves at the dropping mercury electrode in ammoniacal solutions containing cobaltous chloride ; the wave found with αα′-dimethylcystine was likewise indistinguishable from that given by cystine when the two amino acids were compared at a concentration of $7\times10^{-6}$M in a mixture containing 0·1M $NH_4Cl$, 0·1M $NH_4OH$, and 0·002M cobaltous chloride, and this wave could clearly be made the basis of an analytical method. A polarographic determination of αα′-dimethylcystine has been reported recently by Thibert and Ottenbrite (1960) who showed that the diffusion current in 0·1N HCl (containing thymol as a maximum suppressor) is proportional to amino acid concentration in the range $5\times10^{-4}$ to $2\times10^{-3}$M.

Comparison of the stabilities of cystine and αα′-dimethylcystine to a wide range of boiling alkaline solutions (see Section III) showed clearly that the new amino acid is considerably more stable than cystine. Furthermore, cystine dimethyl ester is decomposed in methanolic triethylamine in the cold, with formation of elemental sulphur, while αα′-dimethylcystine dimethyl ester is stable to this reagent even at the boiling point. On the other hand, both disulphides are decomposed readily by silver nitrate in neutral ammonium acetate solution at 42 °C, are also attacked slowly by mercuric chloride in a potassium chloride–borate buffer at pH 9, and give positive thiol tests after treatment with sulphite or cyanide, showing that the α-methyl groups do not hinder direct reaction at the sulphur atoms when this is the usual mode of action. These results therefore support the hypothesis that the alkaline decomposition of cystine is to a large extent the result of attack by a nucleophile at the amino acid α-hydrogen atom, with displacement of this proton and β-elimination of disulphide anion, $RSS^-$. However, the fact that αα′-dimethylcystine *is* decomposed in boiling alkali, even though much more slowly than cystine, indicates that reaction pathways other than β-elimination are possible and these alternative mechanisms could play a secondary role in the decomposition of cystine and its derivatives.

Hydrodisulphides, RSSH, the proposed intermediates in β-elimination, have been very little studied. Gutmann (1915) showed that sodium ethyl-

thiolsulphate reacts with potassium hydrogen sulphide to give a solution which almost certainly contains ethyl hydrodisulphide,

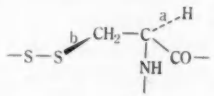
$$C_2H_5SSO_3Na + HS^- \rightarrow C_2H_5SS^- + NaHSO_3,$$

while Rao and Gorin (1959) have obtained evidence that a hydrodisulphide anion is formed in the reduction of cystine with sulphide solutions,

$$RSSR + S^{--} \rightarrow RSS^- + RS^-.$$

Böhme and Zinner (1954) have prepared authentic ethyl- and benzylhydrodisulphide, and shown that these compounds decompose readily on heating to give the corresponding thiols and elemental sulphur. The formation of one equivalent of sulphur during the decomposition of cystine dimethyl ester in triethylamine–methanol can therefore be plausibly explained on the basis of hydrodisulphide decomposition.

If the β-elimination hypothesis is accepted, it becomes of interest to consider the stereochemical consequences for the action of alkali on proteins. It is known that for a wide range of classical β-eliminations, reaction is facilitated if the two bonds which are broken, shown *a* and *b* in II, are initially in a *trans*-configuration to the bond which remains as a carbon–carbon double bond at the end of the synchronous process (see e.g. Ingold 1953):




(II)

In a protein, therefore, stability to alkali would be enhanced if the bonds *a* and *b* of the combined cystine residues were in a *cis*- rather than *trans*-configuration to the $C_\alpha$—$C_\beta$ bond, and for proteins where wide differences in reactivity between different disulphide groups have been observed, the differences may be partly due to conformational factors involving the α- and β-carbon atoms of the amino acid residues.

## III. Experimental

Melting points are uncorrected. Analyses are by the C.S.I.R.O. and University of Melbourne Microanalytical Laboratory.

### (*a*) *Synthesis of Amino Acids*

(i) *5-(Benzylthiomethyl)-5-methylhydantoin.*—Starting with pure 1-(benzylthio)acetone (Wahl 1922) (18·5 g) and following the directions of Potts (1955) the product (21·9 g, 85%) was obtained as needles, m.p. 119 °C, after one recrystallization from ethanol. The hydantoin could also be prepared from bromoacetone without isolation of the intermediate ketone, as follows: Metallic sodium (13 g) was added in small pieces to toluene-ω-thiol (69 g) in ethanol (200 ml) with occasional cooling. To the clear solution bromoacetone (75 g) was added slowly with cooling in ice; the mixture was then kept for 1 hr at room temperature and the sodium bromide filtered off. The filtrate was added to a slurry of ammonium carbonate (162 g) and potassium cyanide (48 g) in water (200 ml) and the mixture heated with stirring in an open vessel at 65–75 °C for 7 hr. The mixture was then heated on a steam-bath to remove any remaining alcohol and the aqueous layer was acidified (caution: $CO_2$, HCN). The brown viscous oil solidified on cooling and was then ground with water, filtered off, washed well to remove soluble salts, and dried in air. Yields

from three experiments were 84 g (m.p. 105–110 °C), 115 g (m.p. 95–100 °C), and 97 g (m.p. 90–100 °C). The crude product was best purified as follows: A 20 g portion was boiled with water (1 l.), the solution was decanted from the dark brown insoluble oil, cooled, the crystals removed, and the filtrate used to treat further batches of crude material. An overall yield of 36% was obtained.

(ii) *S-Benzyl-α-methyl-*DL*-cysteine.*—This was prepared from the above hydantoin exactly as described by Potts (1955), except that care was taken to extract the precipitated barium carbonate a number of times with *boiling* water. The amino acid is either very strongly adsorbed to barium carbonate or else is present in part as the barium salt of the corresponding carbamate, which is decomposed on heating. The product had m.p. 228 °C (decomp.), Potts gives m.p. 234 °C, Connors and Ross (1958) m.p. 237–238 °C, and Arnstein (1958) m.p. 244 °C. It is advantageous to wash the final product with warm ethanol to remove any unchanged hydantoin.

(iii) (±)-*αα′-Dimethylcystine.*—*S*-Benzyl-α-methyl-DL-cysteine (10 g) in liquid $NH_3$ (400 ml) was treated with metallic sodium (4–5 g) until the blue colour was stable for several minutes. $NH_4Cl$ (8–9 g) was added and the $NH_3$ allowed to evaporate. The residue was dissolved in water (200 ml), extracted with ether (2 × 100 ml), filtered, and aerated until the nitroprusside test was negative (2 days). After making just acid the solution was applied to a column of " Zeokarb-225 " (or " Dowex-50 ") resin in the $H^+$ form and washed with water until all chloride was removed. The *amino acid*, presumably a mixture of DL- and *meso*-forms, was eluted with 1N $NH_4OH$ and the solution evaporated, giving 4·4 g (74%) of product, decomposing at 248–250 °C, unchanged on crystallization from aqueous ethanol (Found: C, 35·4; H, 6·0; N, 9·9; S, 23·4%. Calc. for $C_8H_{16}O_4N_2S_2$: C, 35·8; H, 6·0; N, 10·4; S, 23·9%). The amino acid has been prepared independently by Arnstein (1958), who gives m.p. 260 °C (decomp.). The acid readily gives supersaturated solutions. Thus when 0·8 g was dissolved in 0·74N $NH_4OH$ (13 ml) and the solution neutralized with 2N acetic acid (5 ml) and kept at 0 °C, no crystals appeared even after 8 days. On seeding, slow crystallization occurred and 0·24 g was filtered off after keeping for 1 month. Like cystine, the new amino acid is strongly yellowed by exposure to ultraviolet light. Esterification in methanol containing anhydrous HCl yielded the *dimethyl ester dihydrochloride*, which crystallized from methanol–diethyl ether, m.p. 200 °C (Found: C, 32·7; H, 6·3; N, 7·3%. Calc. for $C_{10}H_{22}O_4N_2S_2Cl_2$: C, 32·5; H, 6·0; N, 7·6%).

(iv) *α-Methyl-*DL*-cysteine Hydrochloride.*—*S*-Benzyl-α-methyl-DL-cysteine was debenzylated as described in (iii), but the aeration stage was omitted. The weakly acid solution was run through a column of " Zeokarb-225 " resin ($H^+$ form) and the resin washed free of chloride using oxygen-free water. The amino acid was displaced using 1N $NH_4OH$ prepared with oxygen-free water, the effluent being collected in 10 ml fractions in a small cup continuously flushed with hydrogen. A tap at the bottom of the cup allowed the effluent to be removed while still maintaining the hydrogen atmosphere. Each fraction was at once evaporated to dryness. When the amino acid appeared it was immediately redissolved in dilute HCl, decolourized with charcoal, and the solution again evaporated to dryness, finally over NaOH in a vacuum desiccator. The product, without further purification, melted at 175–182 °C, was free of sodium and ammonium ions, and had a thiol content greater than 95% theory (Found: C, 27·6; H, 5·9; N, 8·2%. Calc. for $C_5H_{10}O_2NSCl$: C, 28·0; H, 5·9; N, 8·2%). The compound has been prepared independently by Connors and Ross (1958), who purified the amino acid via its mercuric salt and give m.p. 190–195 °C for the hydrochloride crystallized from methanol.

### (*b*) *Comparison of the Alkali Stability of Cystine and αα′-Dimethylcystine*

(i) A mixture of calcium hydroxide (0·66 g), water (20 ml), and a 10% solution of lead acetate trihydrate (7 ml) was heated under reflux in a stream of hydrogen, the effluent gas being passed into a flask containing 4% boric acid (10 ml). At zero time a solution of 0·2 g L-cystine dissolved in 0·25N NaOH (10 ml) was added to the boiling solution, and the $NH_3$ which was evolved was collected in the boric acid and titrated at intervals with 0·1N HCl using bromphenol blue indicator. The percentage decomposition, assuming 2 moles $NH_3$ from each mole of cystine, at times 10, 15, 20, 40, and 60 min was 17·3, 74·7, 85·9, 88·7, and 89·0 respectively. In a similar experiment with αα′-dimethylcystine the decomposition after 40, 120, 180, and 300 min was 1·9, 2·2, 2·6, and 3·0% respectively.

(ii) A solution of cystine or αα′-dimethylcystine ($7 \cdot 5 \times 10^{-4}$M) in 0·25N NaOH was heated under reflux in a stream of hydrogen. Samples (15 ml) were withdrawn at intervals and were acidified with 1N HCl (30 ml). Any $H_2S$ was removed in a stream of hydrogen, and aliquots of the solution were then analysed for thiol and disulphide using the phosphotungstic acid method essentially according to Schöberl and Rambacher (1937–38) (cf. Block and Bolling 1951, p. 193). Results are shown in Table 1.

(iii) A solution of the amino acid ($7 \cdot 5 \times 10^{-3}$M) in 0·1N NaOH was heated under reflux in a stream of nitrogen. Samples were withdrawn and analysed as described under (ii); in addition, the $NH_3$ evolved was collected continuously and titrated with 0·02N $H_2SO_4$. These acid titres were corrected for the amounts of reaction liquor removed for each SH and SS analysis. Results are shown in Table 2.

(iv) Solutions of the two amino acids ($6 \cdot 25 \times 10^{-3}$M) in 0·2M glycine buffer, pH 11·0, were heated under reflux in a stream of nitrogen. After 1, 2, 3, and 4 hr, cystine was decomposed to the extent of 6·1, 12·2, 24·1, and 31·8% respectively. In the corresponding experiment with αα′-dimethylcystine no loss of disulphide was found even after 6 hr.

TABLE 1

DECOMPOSITION OF CYSTINE AND αα′-DIMETHYLCYSTINE IN BOILING 0·25N NaOH

| Amino Acid | Time of Heating (hr) | RSSR Destroyed (%) | RSH formed (mole per mole RSSR present initially) |
|---|---|---|---|
| Cystine .. | 0·5 | 57 | 0·27 |
| | 1 | 89 | 0·39 |
| | 2 | 100 | 0·50 |
| αα′-Dimethyl- | 3 | Nil | Nil |
| cystine | 6 | 16·4 | 0·10 |
| | 13 | 32·8 | 0·31 |
| | 18 | 49·3 | 0·48 |

(v) In an analogous experiment using 0·1M $NH_4Cl$, 0·1M $NH_4OH$, 0·2M glycine buffer, pH 9·5, cystine was decomposed to the extent of 9% after 6 hr refluxing, while there was no detectable loss of αα′-dimethylcystine.

(vi) Samples of cystine or αα′-dimethylcystine (100 mg) and various mixtures of cysteine–cystine and α-methylcysteine–αα′-dimethylcystine were heated for periods ranging from 2–15 hr at 100–110 °C in sealed tubes containing either 0·2N $Ba(OH)_2$ (1 ml), solid $Ba(OH)_2.8H_2O$ (1 g), or 5% $Na_2CO_3$ (25 ml). Barium ions were removed with $H_2SO_4$ and all solutions were finally desalted by bringing the pH to 5 and passing them through a short column of the sulphonic acid ion exchanger "Zeokarb-225", washing the column well with water, and then eluting the adsorbed amino acids with dilute $NH_4OH$. The amino acids present were separated by paper chromatography (downward flow on No. 1 Whatman paper) using the solvent systems A, phenol : water (3 : 1 by wt.); B, *sec.*-butanol : acetic water : water (6 : 1 : 3 by vol), and C, the top layer from a mixture of mesityl oxide : 90% formic acid : water (1 : 1 : 2 by vol) (cf. Thompson 1956). Appropriate controls were included and the spots were located by the usual ninhydrin spray. $R_F$ values for the various amino acids are shown in Table 3.

In the case of cystine heated in 0·2N $Ba(OH)_2$ much decomposition was evident and spots corresponding to cysteic acid and lanthionine, with only very small amounts of unchanged cystine, were detected. In experiments using concentrated $Ba(OH)_2$, large amounts of alanine were also formed. With αα′-dimethylcystine, a large amount of unchanged amino acid was detected in

TABLE 2

DECOMPOSITION OF CYSTINE AND αα′-DIMETHYLCYSTINE ($7 \cdot 5 \times 10^{-3}$M) IN BOILING 0·1N NaOH

| Amino Acid | Time of Heating (hr) | Total $NH_3$ evolved (μmole/ml of original solution) | Disulphide destroyed (%) | Thiol produced (mole per mole of disulphide initially present) |
|---|---|---|---|---|
| Cystine .. .. | 0 | — | — | — |
| | 0·5 | 0·17 | 12 | 0·08 |
| | 1·0 | 0·53 | 22 | 0·18 |
| | 1·5 | 0·89 | 33 | 0·29 |
| | 2·0 | 1·27 | 39 | 0·41 |
| | 2·5 | 1·59 | 46 | 0·50 |
| | 4·0 | 2·24 | 68 | 0·70 |
| αα′-Dimethylcystine | 0 | — | — | — |
| | 0·5 | 0·12 | 5·0 | — |
| | 1·0 | 0·28 | 7·0 | 0·02 |
| | 2·0 | 0·44 | 9·0 | 0·06 |
| | 3·0 | 0·66 | 14 | 0·10 |
| | 4·0 | 0·87 | 18 | 0·12 |
| | 5·0 | 1·10 | 20 | 0·19 |
| | 6·0 | 1·40 | 24 | 0·31 |

all cases, together with traces of α-methylcysteic acid and a second substance which from its chromatographic behaviour before and after oxidation with performic acid was probably an intermediate oxidation product ($R_F$ values 0·26 in A, 0·44 in B, 0·26 in C). No new compound which might conceivably have been αα′-dimethyl-lanthionine was detected in any of the experiments.

TABLE 3

$R_F$ VALUES OF SOME AMINO ACIDS ON WHATMAN NO. 1 PAPER

| Amino Acid | Chromatographic Solvent* | | |
|---|---|---|---|
| | A | B | C |
| Cystine .. .. .. .. | 0·36 | 0·10 | 0·04 |
| αα′-Dimethylcystine .. .. | 0·58 | 0·27 | 0·17 |
| Cysteic acid .. .. .. | 0·04 | 0·01 | 0·03 |
| α-Methylcysteic acid .. .. | 0·15 | 0·01 | 0·06 |
| Alanine .. .. .. .. | 0·58 | 0·43 | 0·37 |
| Lanthionine .. .. .. | 0·25 | 0·08 | 0·05 |
| Lanthionine sulphone .. .. | 0·15 | — | 0·02 |
| α-Amino*iso*butyric acid .. .. | — | 0·50 | 0·48 |

* See Section III (*b*) (vi).

(vii) Cystine dimethyl ester dihydrochloride (0·2 g) and cysteine methyl ester hydrochloride (0·1 g) were dissolved in methanol (10 ml) and triethylamine (1·0 ml) was added. After 2 days at room temperature the solution was a deep yellow-orange colour and after 3 days crystals of sulphur appeared. The sulphur (0·015 g) was filtered off and the filtrate was evaporated to

dryness under reduced pressure. The residue was taken up in 6N HCl, a further small amount of sulphur was removed, and the solution was heated under reflux for 1 hr. Chromatography (see (vi) above) demonstrated the presence of alanine and lanthionine, as well as some unchanged cystine. When the same experiment was repeated, but with omission of the cysteine methyl ester hydrochloride, the products again included sulphur and (after hydrolysis) lanthionine and alanine. When cystine dimethyl ester dihydrochloride (0·3 g) was allowed to stand in a mixture of water (1 ml) and triethylamine (0·4 ml), a white solid separated slowly and was identified as cystine.

(viii) αα′-Dimethylcystine dimethyl ester dihydrochloride (0·1 g) was dissolved in methanol (10 ml) and triethylamine (0·2 ml). The solution was still colourless after 2 weeks at room temperature and no sulphur was formed. Chromatography after hydrolysis showed that the amino acid was unchanged. A similar result was found when the methanol–triethylamine solution was heated under reflux for 24 hr. No sulphur or thiol was formed and the solution remained colourless, but some indications of partial decomposition were found by paper chromatography.

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# SHORT COMMUNICATIONS

## THE CHEMICAL CONSTITUENTS OF AUSTRALIAN *FLINDERSIA* SPECIES*

### XII. THE CONSTITUENTS OF FLINDERSIA XANTHOXYLA DOMIN.

By E. Ritchie,† W. C. Taylor,† and D. V. Willcocks†

*Flindersia xanthoxyla* Domin. (syn. *Flindersia oxleyana* F. Muell.), a large tree ranging from the Richmond River, N.S.W., northward into Queensland, is also commonly known as long jack because of its long clean stem. The timber, which is used in joinery, in shipbuilding, and sometimes in cabinet work, has a light to pronounced yellow colour, which occasions another vernacular name, yellow wood. It is stated by Maiden (1889)‡ to yield a useful yellow dye.

This paper records the results of a systematic extraction of the bark, leaves, and wood. No pigment could be extracted from the wood, but instead a small yield of hesperidin (0·05 per cent.) was isolated. The same substance (0·035 per cent.) was obtained from the leaves but the bark afforded hesperidin (0·1 per cent.), maculine (0·003 per cent.), flindersiamine (0·005 per cent.), and sitosterol (0·02 per cent.).

*Experimental*

Melting points are uncorrected. Light petroleum refers to the fraction of b.p. 60–90 °C. Infra-red spectra were determined in paraffin mulls on a Perkin–Elmer Infracord 137. The substances isolated were identified by direct comparison (mixed m.p.'s and infra-red spectra) with authentic specimens.

(*a*) *Extraction of the Bark.*—The dried milled bark (35 kg) which had been collected at Whian Whian, Queensland (C.S.I.R.O. SN 6020) was exhausted by percolation at room temperature in turn with light petroleum, ether, acetone, and methanol. Each extract was concentrated to about 1000 ml and refrigerated for several days before being worked up.

The light petroleum extract was filtered from a small amount of amorphous material, concentrated further, and the residue taken up in ether. The ethereal solution was shaken with 5% hydrochloric acid (10 × 100 ml) until a negative test was obtained with Mayer's reagent. The aqueous extract was basified with ammonia and extracted with chloroform to yield a crude alkaloid fraction which was combined with similar material from the ether extraction (see below).

The ethereal solution was extracted in turn with 5% sodium bicarbonate (5 × 100 ml), 5% sodium carbonate (12 × 100 ml), and 2% sodium hydroxide (5 × 100 ml). Each extract was acidified and the liberated fractions recycled. All attempts to isolate individual substances from the very dark fractions so obtained (2, 40, and 3 g, respectively) by chromatography on acid-washed alumina or on silica gel, or by chromatography or distillation after methylation with diazomethane, were unsuccessful.

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† Department of Organic Chemistry, University of Sydney.

‡ Maiden, J. H. (1889).—"The Useful Native Plants of Australia." p. 296. (Turner & Henderson: Sydney.)

The remaining ethereal solution was concentrated to a dark brown oil (40 g), which was mixed with methanol (100 ml). After several days the crystalline material (5 g) which had separated was collected and identified after purification as sitosterol.

The methanol filtrate was evaporated and the residue saponified by keeping its solution in 10% aqueous alcoholic potassium hydroxide for 2 days at room temperature. The reaction mixture on working up yielded no crystalline acidic fractions but a further amount of sitosterol (1 g) was obtained by chromatography.

The ether extract was separated into basic, acidic, and neutral fractions as above. The combined light petroleum and ether basic fraction was dissolved in chloroform and the solution passed through a short column of alumina to remove dark impurities. The material (2 g) in the eluate on chromatography on alumina (60 g) yielded maculine (0·31 g) and flindersiamine (0·94 g).

No crystalline material could be obtained from the small acidic and phenolic fractions, but the neutral fraction after saponification gave sitosterol (1·0 g).

The acetone extract deposited a dark brown solid which, after washing with warm ethanol and recrystallization from a large volume of methanol, afforded hesperidin (14 g).

The acetone filtrate was concentrated and the residue shaken with ether and water. The ethereal extract on working up as above yielded maculine (0·18 g) and flindersiamine (0·8 g).

The methanol extract was treated by the same procedure as that used for the acetone extract. Hesperidin (20 g) and maculine (0·46 g) were isolated.

(*b*) *Extraction of the Leaves.*—The leaves (17 kg) were processed by the above methods. The acetone extract gave hesperidin (6 g).

(*c*) *Extraction of the Wood.*—The finely milled yellow wood (6·9 kg) yielded hesperidin (1·2 and 2·3 g) in the acetone and methanol extracts respectively.

The authors are indebted to Mr. W. T. Jones, C.S.I.R.O., Brisbane, for supplying the plant material.

## THE CHEMICAL CONSTITUENTS OF AUSTRALIAN *FLINDERSIA* SPECIES*

### XIII. THE CONSTITUENTS OF FLINDERSIA BENNETTIANA F. MUELL.

By M. N. Galbraith,† E. Ritchie,† and W. C. Taylor†

*Flindersia Bennettiana* F. Muell. is a large tree found in the rain-forests of eastern Australia, ranging from the Clarence River to Maryborough. The wood finds some use in cabinet-making and is known commercially as " Bennett's ash ".

The bark, leaves, and wood have now been systematically extracted and some of the constituents isolated and identified, the results being presented in Table 1. As with several other members of the genus, some of the extracts yielded sizable " acidic " and " phenolic " fractions from which pure substances

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† Department of Organic Chemistry, University of Sydney.

could not be isolated. It may also be noted that the *iso*pimpinellin isolated had the pale yellow colour, described originally by Wessely and Kallab (1931), which extensive purification did not eliminate.

TABLE 1

THE CONSTITUENTS OF F. BENNETTIANA

| Substance | Bark (%) | Leaves (%) | Wood (%) |
|---|---|---|---|
| Osthol .. .. .. | 0·14 | 0·016 | 0·0032 |
| Seselin .. .. .. | 0·039 | 0·0066 | 0·0013 |
| *iso*Pimpinellin .. .. | 0·014 | — | — |
| Flindersiamine .. .. | 0·0020 | 0·00052 | — |
| Skiammianine .. .. | 0·00025 | — | — |
| Maculine .. .. .. | — | — | 0·00065 |
| Sitosterol .. .. .. | 0·010 | 0·0028 | 0·0059 |

## *Experimental*

Melting points are uncorrected. Light petroleum refers to the fraction of b.p. 60–90 °C. The ultraviolet spectrum was measured in purified ethanol with a Hilger Uvispek and infra-red spectra in paraffin mulls with a Perkin–Elmer Infracord 137. The general procedure for extraction and isolation that was followed has been outlined in Part XII of this series (Ritchie, Taylor, and Willcocks 1960). Substances isolated were identified by direct comparison (mixed m.p.'s and infra-red spectra) with authentic specimens.

(*a*) *Extraction of the Bark.*—The bark (26·0 kg) had been collected at Imbil, Queensland (C.S.I.R.O. SN 6033).

The light petroleum extract gave a crude alkaloid fraction which, after being combined with a similar fraction from the ether extract, yielded on chromatography on alumina, flindersiamine (0·37 g) and skiammianine (0·045 g). After removal of acidic and phenolic fractions, the residual dark oil was dissolved in light petroleum (2 volumes) and the solution refrigerated for several days, when crude osthol (24·3 g) separated. The material in the filtrate on cold saponification yielded a neutral fraction, which on chromatography on alumina, afforded sitosterol (0·93 g), and a lactone fraction, which crystallized on scratching. Two recrystallizations from methanol gave osthol (1·82 g). The combined mother liquors were evaporated and the residue chromatographed on alumina. The light petroleum, benzene, and ether eluates yielded colourless crystalline material melting over the range 65–75 °C. Attempts to separate the constituents of this mixture by chromatography, fractional crystallization, or by fractional sublimation or distillation, were unsuccessful. However, when a dilute solution in light petroleum was allowed to evaporate at room temperature a mixture of needles and large prisms formed which it was possible to separate manually. On recrystallization from light petroleum, the needles afforded osthol (2·84 g), and the prisms, seselin (7·68 g). The chloroform eluates contained *iso*pimpinellin which separated from methanol in pale yellow needles (0·21 g) (light absorption: ultraviolet, $\lambda_{max.}$ 223,240–250 (plateau), 270, and 312 mμ; log ε 4·25, 3·98, 4·11, and 3·93 respectively).

The ether extract gave an alkaloid fraction which was combined with that from the light petroleum extract (see above). After removal of the acid and phenolic fractions, the neutral fraction was dissolved in benzene–ether (4 volumes, 5 : 1) and refrigerated for several weeks, when *iso*pimpinellin (1·52 g) crystallized out. The remainder of the material after cold saponification yielded osthol (2·23 g), seselin (0·93 g), and sitosterol (1·43 g).

The acetone extract was concentrated and the residue shaken with ether and water. The ethereal solution on working up in the usual manner gave flindersiamine (0·156 g), skimmianine (0·02 g), osthol (1·72 g), seselin (1·5 g), *iso*pimpinellin (1·87 g), and sitosterol (0·19 g).

The methanol extract was treated in the same way as the acetone extract and afforded osthol (0·54 g), seselin (0·093 g), *iso*pimpinellin (0·07 g), and sitosterol (0·15 g).

(*b*) *Extraction of the Leaves.*—The leaves (16·1 kg) were extracted successively with light petroleum, ether, and methanol. The light petroleum extract yielded osthol (1·08 g) and seselin (0·46 g), the ether extract, osthol (0·83 g), seselin (0·31 g), and sitosterol (0·37 g), and the methanol extract, flindersiamine (0·083 g), osthol (0·73 g), seselin (0·29 g), and sitosterol (0·08 g).

(*c*) *Extraction of the Wood.*—The wood (14·5 kg) was treated as in (*b*) above. The light petroleum extract afforded maculine (0·033 g), osthol (0·35 g), seselin (0·11 g), and sitosterol (0·28 g), the ether extract, maculine (0·058 g), osthol (0·11 g), seselin (0·082 g), and sitosterol (0·57 g), and the methanol extract, maculine (0·004 g).

The authors are indebted to Professor F. Galinovsky and Professor F. Wessely, University of Vienna, for authentic specimens of seselin and *iso*-pimpinellin respectively, and to Mr. W. T. Jones, C.S.I.R.O., Brisbane, for supplying the plant material.

*References*

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## CORRIGENDA

VOLUME 13, NUMBER 1

Page 9, caption to Figure 7: For $x$ read $w$.
Page 17, equation (**A25**): For $(1—m)/m$ read $m/(1—m)$.

VOLUME 13, NUMBER 2

Page 232, Table 2, opposite Acetone: *For* item 3227 *read* 3277.

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